Suzan Willemsen

Advanced glycation end-products in chronic heart failure

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Stellingen

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Advanced glycation end-products in chronic heart failure

door
Suzan Willemsen

1. De spiegels van versuikerde eiwitten zijn gerelateerd aan diastolische functie en inspanningsvermogen. *(dit proefschrift)*

2. De spiegel van versuikerde eiwitten (AGEs) voorspellen de prognose van patiënten met hartfalen met een behouden en in patiënten met hartfalen met een verminderde linker ventrikel functie. *(dit proefschrift)*

3. Behandeling met de AGE-breaker alagebrium verbetert niet het inspanningsvermogen of de cardiale functie in systolische hartfalen patiënten. *(dit proefschrift)*

4. Behandeling met de AGE-breaker alagebrium verlaagt niet de spiegels van versuikerde eiwitten in het bloed of in de huid. *(dit proefschrift)*

5. Spiegel van versuikerde eiwitten is gerelateerd aan de ontwikkeling van nier en hartfalen. *(dit proefschrift)*

6. There remain plenty of opportunities in an AGE-ing world! *(dit proefschrift)*

7. Een aardige dokter is niet altijd een goede dokter, maar een goede dokter is wel vaak een aardige dokter.

8. Onderzoek is wat ik doe wanneer ik niet weet wat ik aan het doen ben – Werner von Braun.

9. Onderschat nooit de reinigende werking van een theedoek.

10. Duik erin en zie waar je boven komt.

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Voor Joke en Eddy

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Introduction

Introduction

Chronic heart failure (HF) is a complex clinical syndrome resulting from any structural or functional cardiac disorder impairing the ability of the ventricle to fill with or to eject blood combined with symptoms of dyspnoea or fatigue.(1) HF prevalence increases fast due to an aging population and an increasing diabetes prevalence.(1,2) In 70-80 year old people the prevalence of HF is between 10 and 20 %.(1) HF causes 5% of acute hospital admissions and is present in 10% of hospitalized patients. Despite new therapies, the mortality rate of HF is 50% within four years after discovery, and 40% of hospitalized HF patients have either died or are readmitted within one year.(1)

Chronic HF patients may have a preserved (diastolic HF) or depressed (systolic HF) left ventricular ejection fraction (LVEF). Between diastolic and systolic dysfunction, both similarities and differences exist. In both systolic and diastolic HF, ventricular remodeling is an important mechanism for the initiation and progression of HF.(3) However, characteristics of the remodeling features are different in the two HF types.(3) Systolic HF patients have abnormalities in the pressure-volume relationship during systole, including decreased LVEF, stroke volume and stroke work, which causes eccentric hypertrophy.(4) Nevertheless, almost all systolic HF patients also have abnormalities in diastolic function.(4) In fact, diastolic function is even more impaired in systolic compared to diastolic HF patients.(5,6) Diastolic HF patients have decreased ventricular relaxation, abnormal active relaxation, and/or an increase in ventricular and arterial stiffness, causing concentric hypertrophy.(4,7,8) Several mechanisms underlying diastolic HF have been proposed.(9-13) One possible mechanism could be an increase in advanced glycation end-products (AGEs). The current thesis will focus on the role of AGEs in HF patients

Current treatment of heart failure

The purpose of treating HF patients is to improve symptoms and to reduce mortality risk and HF hospitalization.(1) Next to this, the prevention of progression of HF remains an essential part of the treatment.(1) Since systolic and diastolic HF have different remodeling features, treatment targeting underlying mechanisms are different.(13) Among systolic HF patients, several interventions have been well established and survival improved significantly over time.(1) On the other hand, for diastolic HF patients, no successful interventions have demonstrated to improve survival.(14) Therefore, in a 15-year period, the amount of HF patients admitted with reduced LVEF did not change significantly, whereas the amount of HF patients admitted with preserved LVEF increased.(14)

Patients with diastolic HF can theoretically benefit from four different kinds of treatments.

First, it can be expected that symptomatic diastolic HF patients will benefit from diuretics, by reducing fluid overload, and thereby reducing HF symptoms.(15)

Second, any agent with heart rate lowering and negative inotropic characteristics, such as (some) calcium blockers and β-blockers, can increase left ventricular filling time and thereby improve decreased relaxation present in diastolic HF patients.(15)

Third, every blood pressure reducing agent may reduce left ventricular hypertrophy and therefore improve relaxation. As expressed before this relaxation is beneficial for diastolic HF patients.(13)

Fourth, blockers of the renin-angiotensin system will theoretically reduce both pre- and afterload, left ventricular hypertrophy, interstitial collagen deposition and fibrosis as described in literature.(15) Next to this, those blockers may potentially have a favorable effect on relaxation.(15) Examples of these blockers are angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs) and aldosterone antagonists. However, ARBs and ACE-inhibitors have shown no clear beneficial effect in previous clinical studies (6,16-19), although a substudy from SENIORS demonstrated that nebivolol may possibly be equally effective in elderly HF patients with either preserved or impaired ejection fraction.(20) Spironolactone, on the other hand, improved peak exercise VO_2, quality of life and New York Heart Association class in 11 women with diastolic HF.(21) To verify these results, larger randomized trials of aldosterone antagonists in diastolic HF are currently ongoing (for example the Spironolactone for Failure in the Elderly (SPIFFIE) and the Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist (TOPCAT)).

In conclusion, we currently lack diastolic HF agents specifically targeting the underlying mechanisms altered by disease processes.(13) These altered underlying mechanisms can be caused by neurohormonal and cardiac activation and/or inhibition, changes in calcium homeostasis and an increase in collagen in the extracellular matrix.(13) An ideal therapeutic agent should target these mechanisms.

Advanced glycation end products

Elevated levels of advanced glycation end-products (AGEs) can trigger or worsen diastolic HF. AGEs are formed in a non-enzymatic reaction between proteins and sugar residues (a Maillard reaction).(9,22,23) AGEs accumulate in the body during ageing, and are also increased in patients with chronic systolic, or diastolic HF, diabetic complications and/or renal dysfunction.(9,23,24) Furthermore, tissue AGEs are independently associated with diastolic dysfunction.(24) Diastolic dysfunction might be caused by crosslinking capabilities of AGEs and/or the binding capacity of AGEs to the receptor of AGE (RAGE). Upon interaction of AGE with RAGE, an increase in inflammation and fibrosis occurs, causing cardiovascular dysfunction. AGE-accumulation can cause upregulation of RAGE, resulting in an increase in AGE-binding capacity. This creates a potential vicious circle.(25) However, RAGE has a secretory isoform, soluble RAGE (sRAGE), which has been proposed to bind up AGEs and interferes with AGEs' ability to bind and activate RAGE, and thereby slow the progression of inflammation and fibrosis.(26-29)

Elevated AGE levels are associated with a reduced survival rate for patients with diabetes, renal failure, HF or a combination of these comorbidities. Elevated AGE levels in a (HF) patient may therefore be target for intervention. Several methods are demonstrated to intervene on elevated AGE levels. The first method reduces the formation process of AGEs by blocking receptor sites using angiotensin II type 1 receptor blockers (ARBs). The effectiveness of this mechanism has been demonstrated in in vitro and in vivo studies.(30-33) ARBs prevent the production of reactive carbonyl compounds (RCOs), which are critical precursors of AGEs.(30-32) However, conflicting data of the effectiveness of ARBs on AGE accumulation have been presented.(30,32,33) The second method reduces AGE levels by decrease of AGE intake, low-AGE diets and smoking cessation.(9,34,35) In the third method, AGE crosslink breakers might be able to reverse excessive AGE-induced crosslinks in the extracellular matrix. By breaking these crosslinks, structural changes that are related to diastolic dysfunction might be reversed.(36) AGE-crosslink breakers efficacy in HF patients is demonstrated in experimental work and an open label clinical study.(37-41)

In this open label study of 23 elderly diastolic HF patients, 210 mg alagebrium twice a day for 16 weeks reduced left ventricular mass and improved diastolic function. This seems to prove the beneficial effects of AGE-crosslink breakers for HF patients, but this study was only small-scale and only uncontrolled data are available.

Aim of this thesis

The aim of this thesis is to further investigate the role of AGEs, and the effect of the AGE-crosslink breaker alagebrium, in chronic HF patients. This role is clarified in the following chapters.

In **chapter 2** an overview is provided of the physiology of AGEs, the pathophysiological role that AGEs might play in the development and progression of HF and the role of AGEs as a possible pathophysiological factor that link the development and progression of heart and renal failure. This chapter concludes with the role of AGE intervention as a possible treatment for HF patients.

Chapter 3 provides the study design and baseline characteristics of the BENEFICIAL trial, a double-blind, placebo-controlled, randomized proof-of-concept trial evaluating the efficacy and safety of the AGE crosslink breaker alagebrium (alt-711) in chronic HF patients.

In **chapter 4**, the main results of the BENEFICIAL study are presented. AGEs have been associated with diastolic dysfunction through the formation of collagen crosslinks in the heart. Currently no standardized treatment exists to improve diastolic function. AGE-breakers might be able to reverse structural changes that are related to diastolic dysfunction. However, only experimental and open label studies have been performed. The BENEFICIAL study is the first prospective, randomized, double-blind, placebo controlled study to investigate the effects of the AGE-breaker alagebrium, 200 mg twice a day, on exercise tolerance in patients with HF with a reduced LVEF.

Chapter 5 shows the effects of the AGE crosslink breaker alagebrium on plasma AGEs. Alagebrium is supposed to cleave AGE-derived proteins. After AGE crosslinks have been removed, AGEs may shift to plasma, potentially increasing plasma AGEs. Accumulation of plasma AGEs can cause upregulation of sRAGE.(4,9,26) This chapter shows whether alagebrium reduces tissue AGE levels and increases plasma and sRAGE levels in systolic HF patients treated with alagebrium.

In **chapter 6**, the possible association among tissue AGEs, diastolic function and aerobic exercise capacity in HF patients with and without diabetes is being studied. Previously, increased AGE-accumulation has been reported in diabetic patients and in HF patients. Diabetics have a more impaired diastolic function and reduced exercise capacity compared with non-diabetics. Whether this is also applicable for diabetic HF patients is clarified in this

chapter.

Finally, **chapter 7** demonstrates the predictive value of plasma AGEs, N^{ε}-(carboxymethyl) lysine (CML) and pentosidine, in mortality and HF hospitalization and the combined primary endpoint (mortality and HF hospitalization), studied in a large HF population. In a small group of systolic HF patients, CML, after adjustment for renal function, was not related to prognosis.(10) Another study demonstrated the relation between pentosidine and the severity of HF.(42) However, this study was not adjusted for important potential confounders, such as haemoglobin and gender. Therefore, in this chapter, the independent predictive value of CML and pentosidine was studied in a large group of HF patients.

This thesis concludes with a discussion, reflecting the results of this study to the knowledge of AGEs and the AGE-breaker alagebrium in literature and providing future perspectives.

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Advanced glycation end-products, a pathophysiological pathway in the cardiorenal syndrome

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Adriaan A. Voors; *Accepted Heart Failure Reviews*

Abstract

The prevalence of heart failure (HF) is increasing. A distinction is made between diastolic HF (preserved left ventricular ejection fraction (LVEF)) and systolic HF (reduced LVEF). Advanced glycation end-products (AGEs) are crystallized proteins that accumulate during ageing, but are particularly increased in patients with diabetes mellitus and in patients with renal failure. Through the formation of collagen crosslinks, and by interaction with the AGE-receptor, which impairs calcium handling and increases fibrosis, AGE-accumulation has pathophysiologically been associated with the development of diastolic and renal dysfunction. Interestingly, diastolic dysfunction is a frequent finding in elderly patients, diabetic patients and in patients with renal failure. Taken together, this suggests that AGEs are related to the development and progression of diastolic HF and renal failure. In this review, the role of AGEs as a possible pathophysiological factor that link the development and progression of heart and renal failure, is discussed. Finally, the role of AGE intervention as a possible treatment in HF patients will be discussed.

Introduction

The prevalence of chronic heart failure (HF) increases fast due to a population of increasing age and an increasing prevalence of diabetes, resulting in a prevalence of HF of 10-20% in 70-80 year old people.(1,2) Chronic HF may occur in the presence of a preserved (diastolic HF) or depressed (systolic HF) left ventricular ejection fraction (LVEF), both having a similar (poor) prognosis.(1,3-5) The prevalence of diabetes in systolic HF is estimated at 23% (6,7) and in diastolic HF at 25-33%.(8-11) A possible mechanism underlying diastolic HF may be an increase in advanced glycation end-products (AGEs). AGEs are formed during a non-enzymatic reaction between proteins and sugar residues.(12,13) AGEs accumulate in the body with age and are increased in patients with chronic systolic and diastolic HF, diabetic complications and renal dysfunction.(12,14) In diabetic HF patients tissue AGEs are more increased compared with HF patients without diabetes.(14) Whether a difference in accumulation of AGEs in diabetic patients between systolic and diastolic HF is present, remains to be established. AGEs can also activate the receptor for AGE (RAGE) and thereby induce cardiovascular dysfunction.(12) In cardiovascular disease, renal dysfunction often exists and is frequently referred to as the cardiorenal syndrome.(15) The cardiorenal syndrome is a disorder of the heart and kidneys, whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other and vice versa.(16) Interestingly, patients with renal dysfunction often have diastolic dysfunction, and have an increased prevalence of HF, in particular diastolic HF.(17-20) In addition, the risk factors for developing renal dysfunction have an overlap with the risk factors for the accumulation of AGEs.

Cardiorenal syndrome

In patients with chronic HF the co-existence of renal dysfunction is common, and renal failure is among the strongest predictor of mortality in patients with HF.(21) This co-existence has often been referred to as "cardiorenal syndrome", in which acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ.(16,22) The pathophysiology of the cardiorenal syndrome is multifactorial and involves decreased renal perfusion, atherosclerosis and inflammation, endothelial dysfunction and neurohormonal activation.(23,24)

Advanced glycation end-products

Advanced glycation end-products (AGEs) are a heterogeneous group of compounds, formed by oxidative and non-oxidative reactions between proteins and sugar residues, called the Maillard reaction.(12,13) The Maillard reaction is a slow reaction and initiates when protein amino groups are exposed to sugar adducts, and proceeds from reversible Schiff base adducts to more stable, slowly reversible Amadori products (e.g. HbA1c). It further proceeds through the re-arrangement of Amadori products to the formation of stable and irreversible AGE compounds, for example N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(carboxyethyl) lysine (CEL), and pentosidine.(12,13) The final step is catalyzed by oxidative stress, defined as a high steady state level of reactive oxygen species (ROS), which causes an increase in AGEs.(12) This increase in AGEs causes acceleration of oxidation, creating a vicious circle. Rapid formation of AGEs occurs via another pathway involving reactive carbonyl compounds (RCC) during oxidative stress.(25) RCCs are produced from lipids or carbohydrates reacting with ROS. AGE accumulation in vivo occurs throughout the body, including the skin, neural, vascular and renal tissue.(26,27) Smoking cigarettes, heated, cooked or roasted food products are possible sources of increased AGE accumulation.(12,28,29) AGE degradation products are excreted via the kidney.(25)

AGE accumulation can be measured in blood and in tissue. In blood, the preferred technique for determination of CML and CEL is stable-isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS).(30) For determination of pentosidine in blood, a rapid and sensitive high-performance liquid chromatography (HPLC) method is considered the preferred technique.(31) In skin tissue, AGE accumulation can be measured at the volar side of the lower arm, more simple and non-invasively with a skin auto-fluorescence (AF) reader (AGE-reader).(32)

Advanced glycation end-products in diabetes mellitus

Accumulation of AGEs depends on both sugar concentration and the rate of protein turnover.(33) Diabetic patients have a higher sugar concentration and therefore a higher amount of AGEs compared to healthy controls. The rate of formation of Amadori products is directly proportional to the glucose concentration.(33) It has also been shown that diabetes is a risk factor for the development of a more impaired diastolic function, independent of age.(34-36) Furthermore, diabetes is not only increasing the risk of developing HF, but also accelerates its occurrence.(37) This could be partly explained by a higher amount of AGEs, which also occurs in HF patients.(35) Interestingly, diabetics also have a more impaired diastolic function compared with non-diabetics.(34,36) We recently provided evidence for an association between tissue AGEs and diastolic dysfunction (measured with mean E'), suggesting that AGEs might explain diastolic dysfunction in diabetic HF patients.(14)

The RAGE axis

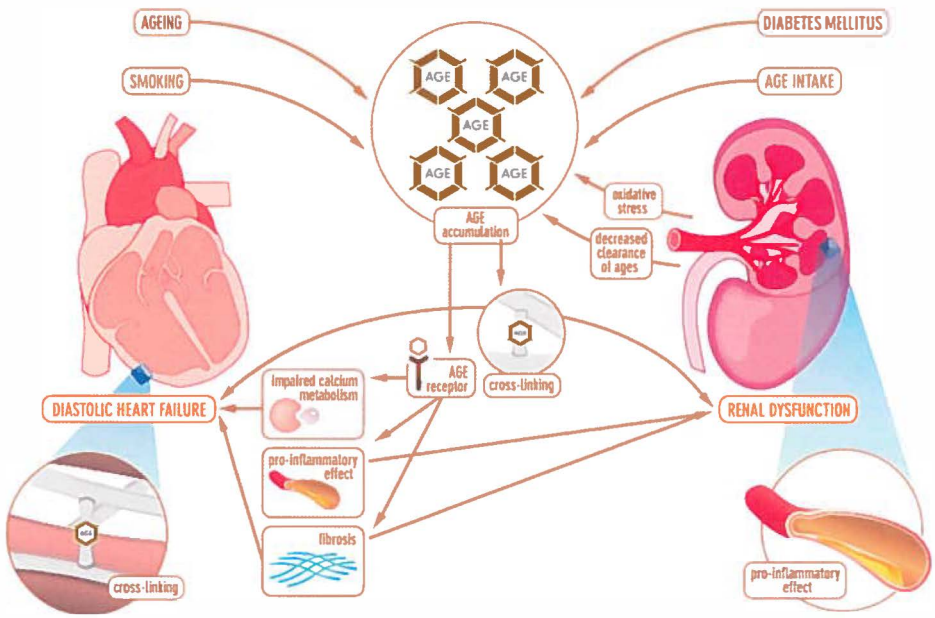
AGEs can also bind to the receptor of AGE (RAGE) and thereby induce cardiovascular dysfunction.(12) RAGE is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules that is expressed in a variety of cell lines.(38) RAGE has a C-truncated secretory isoform, soluble RAGE (sRAGE), that circulates in plasma.(39) sRAGE has been proposed to have an atherosclerotic-protective function, in particular by acting as a decoy for AGE.(39-41) AGE-accumulation can cause upregulation of RAGE.(42) When AGE is interacting with RAGE, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is activated, causing activation and inflammation of NF-kappaB, which induces further inflammation and can increase AGE-accumulation, creating a vicious cycle.(42)

Advanced glycation end-products in heart failure; pathophysiology

Patients with diastolic HF have decreased ventricular relaxation, abnormal active relaxation, and/or an increase in ventricular and arterial stiffness.(5,43,44) The degree of diastolic dysfunction is correlated with the severity of myocardial fibrosis.(45) Several other mechanisms underlying diastolic HF have been proposed.(12,46-49) One possible mechanism could be an increase in AGEs. AGEs may induce diastolic dysfunction in the heart through three different pathways (figure 1). First, AGE accumulation causes excessive cross-linking, which increases rigidity, thereby causing diastolic dysfunction as well as renal dysfunction. Second, when AGEs interact with the AGE receptor, the receptor is activated.

Activation of AGE receptors (most important receptor is Receptor for AGE (RAGE)) causes increased fibrosis via the upregulation of transforming growth factor-ß (TGF- ß). Third, when the AGE receptor is exposed to AGEs, it causes a significant delay in calcium reuptake. As a consequence, the duration of the repolarisation phase of the cardiac contraction may increase, subsequently causing diastolic dysfunction. Based on these data it can be hypothesized that AGEs are a causal factor in diastolic HF.

Figure 1 Schematic representation of the pathophysiological pathways by which advanced glycation end-products causes diastolic heart failure and renal dysfunction



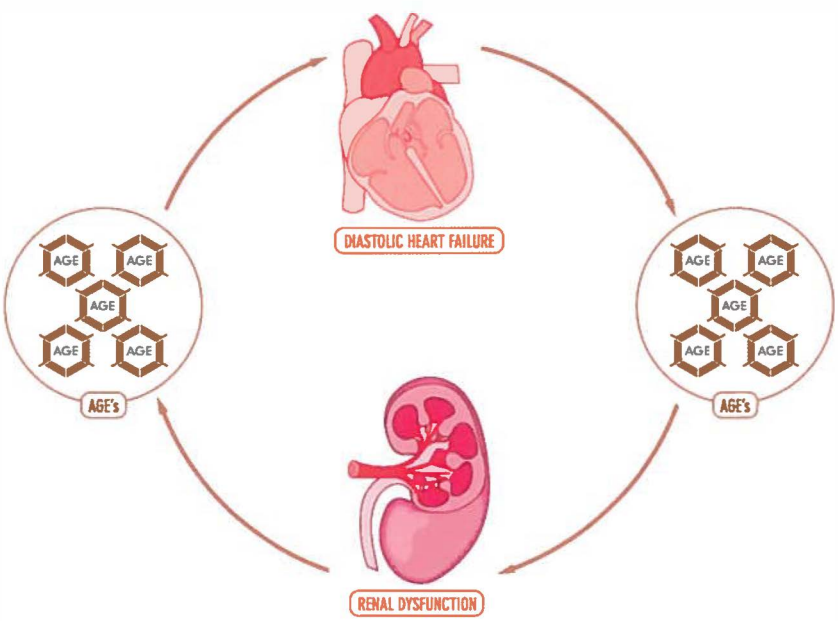
Advanced glycation end-products in heart failure; evidence

Evidence for a role of AGEs in HF patients comes from experimental work and increasing number of clinical studies. In diabetic obese rats diastolic function measured by cardiac catheterization has been shown to correlate with levels of CML.(50) AGE receptor activation influences calcium metabolism and thereby induces diastolic dysfunction. In transgenic mice that overexpressed human RAGE in the heart, diastolic and systolic intracellular calcium concentration was reduced.(51)

We recently showed that tissue AGEs are related to diastolic function in dialysis patients. (18) In another clinical study, we found an association between tissue AGEs and diastolic function (measured with mean E').(14)

The overall prevalence of HF is increasing. Furthermore, roughly 50% of patients die in four years.(1) Several factors have been established as independent predictors for survival in HF patients, among which are LVEF, New York Heart Association (NYHA) functional class, anemia and renal function.(46,52-54) Four studies investigated the prognostic role of AGEs in HF patients. We have previously shown that plasma AGE CML was related to the severity and prognosis of 102 HF patients, but after correction for renal function this relation subsided.(46) In a second study in 141 HF patients, pentosidine was related to severity of HF and it was an independent risk factor to predict adverse clinical outcome.(55) However, the authors did not adjust their findings for all known risk factors for mortality, such as hemoglobin and gender. Furthermore, they may have introduced a possible co-linearity problem by simultaneously introducing creatinine levels and estimated glomerular filtration rate (GFR) in the multivariable model. A third study showed that pentosidine was an independent predictor for cardiac events in 160 HF patients.(56) Cardiac events were defined as a composite end point of cardiac death and rehospitalization.

Figure 2 Advanced glycation end-products and cardiorenal syndrome



Advanced glycation end-products and renal failure; pathophysiology

Several mechanisms underlying renal dysfunction have been proposed, for example decreased renal perfusion, atherosclerosis and inflammation, endothelial dysfunction and neurohormonal activation.(24,57-59) Another possible mechanism could be an increase in AGEs. AGE-accumulation in patients with renal dysfunction can occur through two different pathways (figure 1). First, increased AGE accumulation is caused by decreased clearance of AGE degradation products. After modification or degradation in proximal tubuli, AGEs are eventually cleared in the urine.(60-62) Patients with renal dysfunction have a decreased clearance and thereby accumulation of AGEs occurs. Second, oxidative stress is enhanced in patients with renal dysfunction, which also causes an increase in AGE formation.(63) An increase in AGEs causes acceleration of oxidation, regenerating another increase in AGEs. When endothelial cells in the kidney are stimulated by AGEs, they release the pro-inflammatory mediators vascular cell adhesion molecule-1 and intercellular adhesion molecule-1.(61,64,65) This may lead to tissue damage in the kidney. In summary, it can be hypothesized that AGEs are a possible mechanism underlying renal dysfunction.

Advanced glycation end-products and renal failure; evidence

Evidence for a role of AGEs in patients with renal failure comes from experimental and clinical studies. In 50 healthy male Sprague-Dwaley rats AGEs were increased by adding AGE-modified albumin. This resulted in structural changes in the glomerulus.(66) In healthy nondiabetic rats and rabbits administrating AGE-modified albumin led to an increase in vascular permeability and inflammation in the vasculature.(66) In clinical studies, AGEs accumulate during renal failure and dialysis.(61,67) In patients with renal failure plasma AGEs are elevated.(67,68) In patients on dialysis, tissue AGEs are even more increased compared to patients with renal failure without dialysis.(69) After renal transplantation AGE accumulation is lower than during hemodialysis, but remains elevated.(69)

Renal dysfunction is strongly associated with a poor clinical outcome in HF patients.(54,70-72) In addition, AGEs are related to the severity of HF and its clinical outcome as well. The prognostic role of AGEs in end-stage renal disease has however been inconsistent.(73-76) Two studies reported that high level of CML were associated with an increased mortality, but a third study showed an association with a decreased mortality. However, AGEs are a heterogenous group of compounds. Several different AGEs exist; some show cross-linking properties (pentosidine), whereas others do not (CML and CEL). All three

studies only measured accumulation of the non-cross-linking AGE CML. The cross-linking AGE pentosidine was not measured. Furthermore CML was not measured with LC-MS/MS, which is the preferred technique with highest specificity. Another study reported that tissue AGE was an independent predictor of mortality and associated with cardiovascular disease in hemodialysis patients.(77) Further research is warranted to provide more insight into the prognostic role of AGEs in patients with renal dysfunction.

AGEs as a pathophysiological factor in cardiorenal syndrome

The data presented in this review suggests that AGEs may be involved as a pathophysiological factor in cardiorenal syndrome (figure 1 and 2). AGE-accumulation is not restricted to specific patient groups and accumulates in the body with age. With ageing, diastolic function impairs, while systolic function remains unchanged.(1) Diastolic dysfunction can be caused by AGEs through increasing rigidity and by causing a delay in calcium reuptake.(12) Patients with renal dysfunction are known to have increased AGE-accumulation and diastolic dysfunction is a frequent finding in these patients. The prevalence of diastolic dysfunction in dialysis patients varies from 25-87%, depending on definitions used and patients inluded.(17,20) Diastolic dysfunction predisposes to the development of HF, which causes a further decrease in renal function, creating a vicious circle (figure 1 and 2).(19) Patients with diabetes mellitus are also known to have increased AGE-accumulation, independent of their age (figure 1). Accumulation of AGEs depends on both sugar concentration and the rate of protein turnover. (33) Furthermore, diabetic patients have a more impaired diastolic function compared to non-diabetics.(34,36) Interestingly, the risk factors for developing renal dysfunction have a certain overlap with the risk factors for the accumulation of AGEs.

Taken together, diabetic patients and patients with renal dysfunction have an increased accumulation of AGEs, and an increased prevalence of diastolic dysfunction. In addition, patients with renal dysfunction and diabetes have higher tissue AGE levels compared to patients with renal dysfunction without diabetes.(14,25) Whether this translates into a higher risk for new onset heart failure remains to be established. Together with its pathophysiology, where AGEs can be linked with diastolic dysfunction, we therefore suggest that increased amount of AGEs in heart and renal failure can be a common pathophysiological factor which causes both heart and renal failure (figure 2).

Interventions

AGE-accumulation was associated with a reduced survival in patients with diabetes, renal failure, and HF and may therefore be a target for intervention. The adverse effects of AGE-accumulation can be targeted in several ways.

In vitro and in vivo studies have shown that angiotensin II type 1 receptor blockers (ARBs) can reduce AGE formation.(78-80) ARBs prevent the production of reactive carbonyl and dicarbonyl compounds (RCOs), which are critical precursors of AGEs.(78-81) However, we recently showed that the angiotensin II type 1 receptor blocker eprosartan did not decrease levels of AGEs, within 6 months, in patients with hypertension and diastolic dysfunction.(82)

AGE intake can also increase AGE-accumulation. Both smoking and certain food products contain high levels of AGEs and AGE precursors. Smoking cessation and low-AGE diets have been shown to reduce AGE intake and thereby AGE levels in blood.(12,28,29)

Several AGE crosslink breakers, such as alagebrium and TRC4186, are currently under investigation for use in diabetic and non-diabetic patients. We recently conducted the BENEFICIAL study, the first prospective, randomized, double-blind, placebo controlled study to examine the effects of the AGE-breaker alagebrium on exercise capacity and cardiac function in patients with systolic HF.(83)

Conclusion

There is a pathophysiological and epidemiological link between AGEs, renal dysfunction and heart failure. This suggests that AGEs are related to the development and progression of diastolic HF and renal failure. Therapies targeted at reducing the effects of AGEs will have to provide further evidence for this hypothesis.

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Effects of alagebrium, an AGE breaker, in patients with chronic heart failure: study design and baseline characteristics of the BENEFICIAL trial

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Abstract

Aims

Previous small open label studies have shown that the advanced glycation end-product (AGE) breaker alagebrium may improve cardiac function in patients with chronic heart failure (HF). We report the design, methods and baseline characteristics of a double-blind, placebo-controlled, randomized trial evaluating the efficacy and safety of alagebrium (BENEFICIAL) in patients with HF and a left ventricular ejection fraction (LVEF) ≤ 0.45

Methods and results

Patients with NYHA II–IV stable HF for at least 3 months were eligible for this study. One hundred and two patients were included in the study and randomized to either 200 mg alagebrium twice daily or placebo for a period of 36 weeks. The mean age of patients was 60 ± 11 years, 78% were male, and 17% were diabetic. Mean peak VO_2 was 21.7 ± 5.9 mL/min/kg, mean LVEF was 0.32 ± 0.09. Diastolic function was worse (mean early tissue diastolic velocity (E') 4.6 ± 1.7 vs. 6.1 ± 2.0 cm/s; $P < 0.001$) in patients with LVEF ≤ 0.35 compared to patients with LVEF between 0.35 and 0.45.

Conclusion

The BENEFICIAL study is a proof-of-concept study that will provide new data on the efficacy and safety of the AGE crosslink breaker alagebrium in systolic HF patients. EudraCT number of this trial is NCT00516646.

Introduction

Chronic heart failure (HF) may occur in the presence of a preserved or depressed left ventricular ejection fraction (LVEF).(1,2) Patients with HF with a preserved ejection fraction (diastolic HF) have decreased ventricular relaxation, abnormal active relaxation, and/or an increase in ventricular and arterial stiffness.(2-4) Patients with a depressed ejection fraction (systolic HF) have an abnormal contraction pattern, but the majority of these patients also have diastolic dysfunction.(3,4) In fact, diastolic function is even more impaired in patients with systolic HF compared to patients with diastolic HF.(5) Importantly, in patients with systolic HF, diastolic function, and not systolic function, is related to NYHA HF class and predicts exercise intolerance measured with cardiopulmonary exercise testing.(6)

Even though diastolic dysfunction is common and strongly related to symptoms, there is no standardized treatment to improve diastolic function.(7-9) Previous studies have shown no clear beneficial effect with angiotensin-receptor blockers (ARBs) or angiotensin-converting enzyme (ACE) inhibitors on clinical outcome in patients with diastolic HF,(10-13) although a recent substudy from SENIORS showed that nebivolol may possibly be equally effective in elderly patients with HF with either preserved or impaired ejection fraction.(14) We currently lack HF agents specifically targeted at improving diastolic function. A potential explanation might be that diastolic dysfunction is related tostructural modifications of the extracellular matrix, which can be prevented, but not reversed by ACE-inhibitors, ARBs, or beta-blockers.

Advanced glycation end-product (AGE)-crosslink breakers might be able to reverse the structural changes that are related to diastolic dysfunction (Figure 1). AGEs are end-products formed by oxidative or non-oxidative reactions between sugars and proteins often referred to as the Maillard reaction.(15) AGEs form excessive crosslinks in the extracellular matrix, which may induce myocardial diastolic dysfunction and hypertension.(15-18) Circulating AGEs have been correlated with vascular compliance in humans and may therefore also induce vascular stiffening.(19) Diastolic, systolic, and vascular dysfunction may cause decreased exercise capacity (Figure 1). Therefore, it seems reasonable to believe that AGE-crosslink breakers might improve diastolic function and exercise capacity in patients. This is supported by small and open label studies with alagebrium in patients with diastolic dysfunction.(17,20) In the DIAMOND trial, 210 mg alagebrium twice daily (b.i.d.) given open-label for 16 weeks reduced left ventricular mass and improved diastolic function in 23 elderly patients with diastolic HF.(17) In another open-label study, 35 - 420 mg alagebrium improved diastolic function and left ventricular remodelling in 20 patients with systolic HF, although these results have only been published as an abstract.(20)

Based on the pathophysiological mechanism, supported by small open-label clinical studies, we hereby report the design, methods, and baseline characteristics of a prospective, randomized, double-blind, placebo controlled trial on the effects of the AGE-breaker alagebrium on exercise capacity and diastolic function in 102 patients with systolic HF.

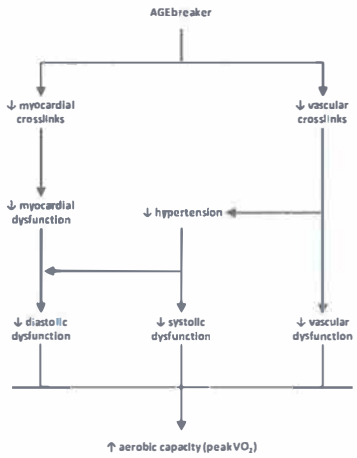


Figure 1 Schematic representation of the pathophysiological pathways by which the advanced glycation end-product breaker alagebrium increases aerobic capacity

Methods

Patients and study design

BENEFICIAL is a prospective, randomized, double-blind, placebo-controlled, phase II study evaluating the efficacy and safety of alagebrium (ALT-711) in patients with HF.

Patients were recruited from the University Medical Center Groningen, the Martini Hospital Groningen, the Refaja Hospital Stadskanaal, and Ommelander Hospital Group location Delfzicht, The Netherlands

Inclusion and exclusion criteria are described in Table 1. In brief, patients with NYHA II – IV stable HF for at least 3 months and LVEF ≤ 0.45 were eligible for the study. Main exclusion criteria were the inability of patients to undergo exercise testing, cardiac resynchronization therapy, pacemaker therapy, active and/or treated malignancies within 12 months prior to inclusion and clinically significant renal dysfunction.

The study schedule is depicted in Figure 2. Patients were randomized to either alagebrium 200 mg b.i.d. or placebo for a period of 36 weeks. Before randomization, a practice cardiopulmonary aerobic capacity test was performed to familiarize the patient with the procedure. Efficacy measurements were performed at baseline, and at the end of the study, and included physical examination, cardiopulmonary aerobic capacity testing

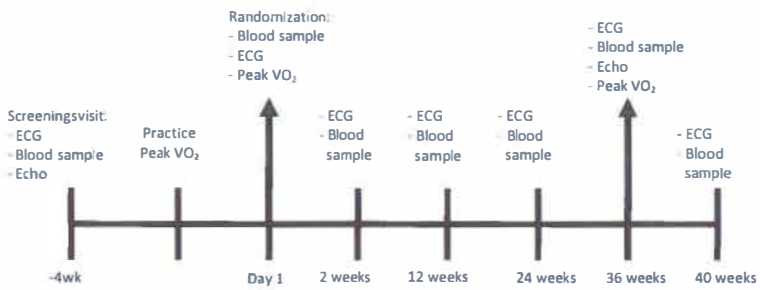
(peak VO_2), echocardiography, Minnesota Living with Heart Failure score, AGE measurements in tissue and in blood, NYHA HF class, patient's and physician's global assessment, and NT-proBNP levels. After randomization, safety visits were performed at 12-week intervals. In addition, one safety visit was performed 2 weeks after the randomization visit and another 4 weeks after study treatment had stopped. At all visits except at the visit to practice the peak VO_2 test, blood pressure, heart rate, skin autofluorescence (skin AF; a validated non-invasive method to study tissue AGEs(21)) were measured, an ECG performed and blood drawn for laboratory analysis. The estimated glomerular filtration rate was calculated using the modification of diet in renal disease (MDRD) formula.(22) Patient's and physician's global assessments were performed at each visit. Recruitment started in November 2007 and was completed at the end of December 2008. The study was approved by the appropriate Medical Ethics Committee and all subjects gave written informed consent

Table 1 Inclusion and exclusion criteria for the BENEFICIAL trial

Inclusion criteria
NYHA II-IV heart failure
Echocardiographic ejection fraction ≤ 0.45
HF duration of at least 3 months
Stable HF medical therapy for at least 1 month
Patients able to understand the study procedures and willing to provide informed consent
Exclusion criteria
Patient aged ≤ 18 years
History of myocardial infarction in previous 6 months
History of stroke/TIA/RIND in previous 6 months
Severe valvular dysfunction
Severe pulmonary disease
History of systemic inflammatory or collagen vascular disease
Active and/or treated malignancies within 12 months prior to inclusion
Any significant condition, either medical or non-medical, that could lead to difficulty complying with the protocol.
Patients on cardiac resynchronisation therapy (CRT) or scheduled for CRT implantation
Pacemaker therapy (unless rescue pacing at ≤ 40 bpm) or scheduled pacemaker implantation
History of valve replacement or surgery
Uncontrolled diabetes (HbA1c > 9.5%)
Clinically significant renal dysfunction (sMDRD calculated GFR ≤ 30 mL/min/1,73m^2)
Clinically significant liver function abnormalities (ASAT/ALAT > 2.5 times the upper limit of normal)
Severe anaemia at baseline (haemoglobin < 10 g/dl or < 6.2 mmol/l)
Use of any investigational drug(s) within 30 days prior to screening
Pregnancy or active breast-feeding (pregnancy tests will be performed on all female subjects of child-bearing potential)
Active pericarditis/myocarditis
Patients unable to undergo exercise testing

NYHA, New York Heart Association; HF, heart failure; MDRD, modification of diet in renal disease; GFR, glomerular filtration rate; TIA, transient ischaemic attack; RIND, reversible ischaemic neurological deficit; ASAT, aspartate amino transferase; ALAT, alanine amino transferase

Figure 2 Study schedule



ECG, electrocardiogram; peak VO_2, cardiopulmonary aerobic capacity test

Skin autofluorescence

Tissue AGE accumulation was assessed using a validated skin AF reader (AGE-reader; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands), as described previously. (21) In short, the AGE reader illuminates an area of skin surface of approximately 2 cm^2, which is protected from the surrounding light, with an excitation light source between 300-420 nm (peak excitation ~ 370 nm). Light from the skin is measured with a spectrometer in the 420-600 nm range, using 200 µm glass fibre. The value of skin AF is calculated as the ratio of the light intensity in the 420-600 nm wavelength range, and the light intensity in the 300-420 nm wavelength range. Skin AF was measured at the volar side of the lower arm at approximately 10-15 cm below the elbow fold. The measurement was performed three times at a healthy skin site (i.e. without visible vessels, scars, or other skin abnormalities) and an average was calculated.

Echocardiography

Two-dimensional echocardiography was performed at the screening visit and at the end of the study (23) by experienced cardiac technicians using a General Electric VIVID 7 system with a 2.5-3.5-mHz probe. Measurements included left ventricular and atrial dimensions. Diastolic function was measured with peak early (E) and late (A) diastolic filling velocities, isovolumetric relaxation time, deceleration time of the early peak filling. Early diastolic tissue velocity (E') was measured on the lateral and septal wall areas, using colour-coded tissue Doppler imaging (TDI). E/E' was calculated by dividing the peak early diastolic filling (E) by the average E'. Diastolic dysfunction was defined as a mean E' < 8 cm/s and/or an E/E' > 10. Systolic dysfunction was determined by Simpson's LVEF and defined as a LVEF ≤ 0.45. If Simpson's LVEF could not be determined, LVEF was estimated visually.

Cardiopulmonary aerobic capacity testing

Cardiopulmonary aerobic capacity testing was performed according to the Modified Bruce protocol, which increases the workload more gradually than the Bruce protocol. The first stage was performed at 1.7 mph and 0% grade, the second stage at 1.7 mph and 5% grade, and the third stage corresponds to the first stage of the Bruce protocol.(24) Each exercise test started with an acclimatization period standing on the treadmill. A standard 12-lead electrocardiogram was recorded continuously during exercise testing. Blood pressure was recorded at regular intervals using a manual cuff sphygmomanometer. Patients were encouraged to continue the exercise until either their peak VO_2 was reached, they became uncomfortably symptomatic, or discontinuation was indicated for safety reasons. Peak VO_2 was determined as an average value of the two highest VO_2 values at peak performance, expressed as ml/min/kg, ml/min/fat-free mass, as well as a percentage of predicted peak oxygen consumption. Oxygen uptake (VO_2), carbon dioxide production (VCO_2), and minute ventilation were measured using breath-by-breath gas analysis.

Endpoints

The primary endpoint of the study is the effect of alagebrium on aerobic capacity measured by cardiopulmonary exercise testing and determined as an average value of the two highest VO_2 values at peak performance expressed as ml/min/kg.(25) Peak VO_2 will also be determined as ml/min/fat-free mass as well as a percentage of predicted peak oxygen consump-tion. Secondary endpoints are: (i) diastolic function measured by TDI; (ii) LVEF measured with echocardiography; (iii) AGEs in tissue measured with skin AF and in blood measured with mass spectrometry analysis; (iv) quality of life measured with Minnesota Living with Heart Failure score; (v) NYHA HF class; (vi) patient's and physician's global assessment scores; and (vii) NT-proBNP levels.

Statistical considerations

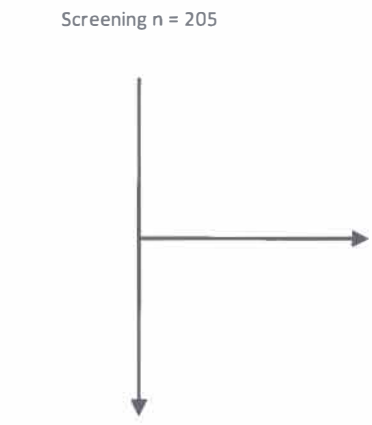
Sample size calculation

The primary aim is to study the effect of alagebrium (ALT-711) on aerobic capacity. According to data from Mancini et al.,(26) the expectation is that an increase of at least 15% in peak VO_2 is clinically significant. In the Mancini study, peak VO_2 increased significantly from 11 ± 0.8 to 12.7 ± 2.8 mL/min/kg. To demonstrate an increase of 15% in aerobic capacity, with a power of at least 80% at a significance level of 0.05, 78 patients would be needed to study the primary objective. With an expected drop-out of 20%, a total of 100 randomized patients will have to be included.

Statistical analysis plan

For safety parameters, all randomized patients who received at least one dose of the study drug will be included in the safety analysis and analysed according to the treatment they actually received. The efficacy analysis will be carried out on the full efficacy analysis (FEA) population. The FEA population consists of all patients who have a baseline measurement and continued medication until at least the 12-week visit. Patients who discontinued before the 12-week visit will not be included in the FEA population. If a patient discontinues at or after the 12-week visit, and can be brought in for assessment within two weeks of stopping study medication, the full 36-week efficacy dataset will be collected and used. All statistical tests will be two-sided. A P-value of less than 0.05 is considered to be statistically significant. Missing data will remain missing, and no attempt will be made to replace missing values. The primary end-point is the absolute change from baseline of the aerobic capacity (peak VO_2) at exercise testing. The effect on the primary and key secondary endpoints will be summarized for subgroups defined by diabetes, age (median), aetiology of HF (ischaemic vs. non-ischaemic), NYHA functional class (II vs. III and IV), gender, peak VO_2, LVEF ≤ 0.35 vs. LVEF > 0.35, mean E', E/E', NT-proBNP, and skin AF. For quantitative parameters, overall group differences will be evaluated using an F-test for normally distributed variables or a Kruskal-Wallis test for variables with a skewed distribution. For qualitative parameters, overall group differences will be evaluated using a χ^2 test.

Figure 3 Reasons for exclusion



Reason for exclusion	Number (n = 103)
LVEF > 0.45	51
Unable to perform peak VO_2	19
Technically insufficient echocardiographic image quality	8
Not in clinically stable condition	8
Ischemia on peak VO_2	7
Unwilling	5
Active malignancy	2
eGFR ≤ 30 mL/min/1,73m²	2
NYHA I	1

Interim analysis

Following randomization of 30 subjects, an interim analysis on the safety of the study drug in the study population will be performed by an independent data safety monitoring board. Investigators remain blinded. Furthermore, safety will be assessed by summarizing incidence and type of adverse events during the study period. All patients will be included in the safety assessment.

Results

A total of 205 patients were screened for the study. One hundred and three patients were excluded (Figure 3). Main reasons for exclusion were LVEF > 0.45 (n = 51), not able to perform a peak VO_2 (n = 19), technically insufficient echocardiographic image quality (n = 8), not in a clinically stable condition (n = 8) and ischaemia on cardiopulmonary aerobic capacity testing (n = 7). A total of 102 patients with stable HF were thus included in the study. Baseline characteristics of the study population are depicted in Table 2. The mean age of patients was 60 ± 11 years, 78% were male, 17% diabetic, and the mean systolic blood pressure was 115 ± 15 mmHg. Mean skin AF was 2.3 ± 0.6 a.u., and mean peak VO_2 was 21.7 ± 5.9 mL/min/kg, which is 84% of the predicted values for patients with similar age and gender. The average calculated early diastolic velocity measured on the lateral and septal wall areas was 5.2 ± 1.9 cm/s, the median E/E' was 12.7 (10.0 - 18.3). Mean LVEF was 0.32 ± 0.09. The median NT-proBNP level was 403 (154 - 851) ng/L. Patients were well treated, 94% of the patients used an ACE-inhibitor or an ARB and 93% a beta-blocker. An important predefined subgroup analysis will be performed on patients with an LVEF ≤ 0.35 (n = 61) and an LVEF between 0.35 and 0.45 (n = 41). Table 2 demonstrates that patients with an LVEF ≤ 0.35 were more often diabetic (14 vs. 3; P = 0.04) and more often had an ischaemic aetiology of HF (47 vs. 23; P = 0.03). Importantly, these patients had more severe HF, as reflected by a lower peak VO_2 (20.4 ± 4.9 vs. 23.5 ± 7.4 mL/min/kg; P = 0.02), higher median NT-proBNP levels (486 (215 - 990) vs. 243 (93 - 567) pg/mL; P = 0.01), and worse diastolic function (mean early tissue diastolic velocity (E') 4.6 ± 1.7 vs. 6.1 ± 2.0 cm/s; P < 0.001).

Table 2 Baseline clinical characteristics of patients

Variables	All patients (n = 102)	LVEF ≤ 0.35 (n = 61)	LVEF > 0.35 (n = 41)	p-value
Age(years)	60 ± 11	61.0 ± 10.8	59.6 ± 12.1	0.54
Sex (male), n (%)	80 (78)	50 (82)	30 (73.2)	0.29
Diabetes, n (%)	17 (17)	14 (23)	3 (7.3)	0.04
Race (Caucasian), n (%)	100 (98)	59 (96.7)	41 (100)	0.24

Table 2 Continued

Variables	All patients (n = 102)	LVEF ≤ 35% (n = 61)	LVEF > 35% (n = 41)	p-value
History of hypertension, n (%)	32 (31.4)	21 (34.4)	11 (26.8)	0.42
Smoking, n (%)				0.19
None	15 (14.7)	8 (13.1)	7 (17.1)	
Current	22 (21.6)	16 (26.2)	6 (14.6)	
Past	65 (63.7)	37 (60.7)	28 (68.3)	
History of hypercholesterolemia, n (%)	58 (56.9)	36 (59)	22 (53.7)	0.59
Body mass index (kg/m²)	28 ± 4.3	28.4 ± 4.4	27.3 ± 4.0	0.20
Systolic blood pressure (mmHg)	115 ± 15	115.9 ± 15.8	113 ± 14.3	0.72
Heart rate (b.p.m.)	69.5 ± 14.3	69 ± 12.3	70.1 ± 17.0	0.39
Aetiology of HF, n (%)				0.03
Ischaemic	70 (68.6)	47 (77)	23 (56.1)	
Non-ischaemic	32 (31.4)	14 (23)	18 (43.9)	
NYHA functional class. n (%)				0.97
II	66 (64.7)	40 (65.6)	26 (63.4)	
III	33 (32.4)	18 (29.5)	15 (36.6)	
IV	3 (2.9)	3 (4.9)	0 (0)	
Laboratory assessments				
eGFR(mL/min/1.73 m²)	79.9 ± 20.8	75.6 ± 19.6	81.9 ± 22.6	0.43
NT-proBNP (ng/L)	403 (154 - 851)	486 (215 - 990)	243 (93 - 567)	0.01
HbA1c (%)	5.7 (5.5 - 6.2)	5.8 (5.6 - 6.3)	5.6 (5.4 - 5.9)	0.03
Total cholesterol (mmol/l)	4.4 ± 1.1	4.3 ± 1.2	4.5 ± 0.99	0.28
Skin AF (a.u.)	2.3 ± 0.6	2.3 ± 0.62	2.1 ± 0.59	0.08
MLHF	18 (7.0 - 29.8)	18 (7 - 29)	18 (7 - 30)	0.79
Echocardiography				
E/A	0.89 (0.69 - 1.17)	0.85 (0.65 - 1.20)	0.93 (0.81 - 1.15)	0.20
Dct (ms)	217.8 ± 57.7	215 ± 63.4	214.4 ± 48.4	0.96
IVRT (ms)	101.6 ± 23.6	104.8 ± 26.0	96.4 ± 18.0	0.07
E/E'	12.7 (10 - 18.3)	14.1 (11.2 - 20.5)	11.0 (9.0 - 13.7)	0.001
Mean E' (cm/sec)	5.2 ± 1.9	4.6 ± 1.7	6.1 ± 2.0	<0.001
LVEF	0.32 ± 0.09	0.26 ± 0.07	0.40 ± 0.03	<0.001
Peak VO_2 (ml/min/kg)	21.7 ± 5.9	20.4 ± 4.9	23.5 ± 7.4	0.02
Percentage of predicted peak VO_2	84 ± 24	79.9 ± 20.5	90.7 ± 30.2	0.05
Medication use, n (%)				
ACE-inhibitors / ARB	96 (94)	59 (97)	37 (90)	0.18
Beta-blockers	95 (93)	58 (95)	37 (90)	0.35
Diuretics	56 (55)	40 (66)	20 (49)	0.09
Aldosterone antagonists	29 (28)	20 (33)	9 (22)	0.24

NYHA, New York Heart Association; MDRD, modification of diet in renal disease; NT proBNP, N-terminal-pro-brain natriuretic peptide; MLHF, Minnesota Living with Heart Failure; Dct, deceleration time; IVRT, isovolumetric relaxation time; LVEF, left ventricular ejection fraction; ACE-i , angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; skin AF, skin autofluo-rescence

Discussion

AGE-breakers might become a novel treatment modality for patients with HF.(27) The aim of the BENEFICIAL study is to evaluate the efficacy and safety of the AGE-breaker alagebrium on aerobic capacity and diastolic function in patients with HF.

We chose to include patients with a decreased LVEF (LVEF ≤ 0.45) for two major reasons. First, we aimed to study the effects of alagebrium in a wide range of HF patients, but since this is a small study, we needed to decrease the risk of including non-HF patients. Therefore, patients should have a clear diagnosis of HF with clear signs of systolic dysfunction. In addition, a subgroup analysis will be performed in patients with LVEF ≤ 0.35 and in patients with LVEF between 0.35 and 0.45. Second, diastolic function is even more impaired in patients with a reduced LVEF, when compared to patients with a preserved LVEF. This is supported by data from our study, in which patients with LVEF ≤ 0.35 had a worse diastolic function than patients with LVEF > 0.35. Interestingly, in patients with HF and a reduced LVEF, diastolic dysfunction (and not systolic function) has been shown to be related to NYHA HF class and to predict exercise intolerance.(5,6) This is a direct result of an increase in left ventricular diastolic pressures and pulmonary venous pressures during exercise in patients with diastolic dysfunction. Therefore, it seems reasonable to believe that exercise intolerance in patients with systolic HF is largely driven by associated abnormal left ventricular diastolic function.

Skin AF was used as a non-invasive measure for skin autofluorescence to assess tissue AGE accumulation. In this population, mean skin AF was 2.3 ± 0.6 a.u. To our knowledge this is the first report of tissue AGE accumulation in patients with HF. Mean age at baseline was 60 years. Furthermore, in this study population 85% of patients are current smokers or were smokers in the past, 31% have hypertension and 17% have diabetes. It is known that AGE accumulation is related to these risk factors.(28)

Expected results and potential clinical implications

Increased AGEs cause ventricular and arterial stiffness through the formation of excessive crosslinks in the extracellular matrix (Figure 1). By breaking these collagen crosslinks, AGE-breakers can reverse a process that seems to contribute to diastolic dysfunction. In contrast, ACE-inhibitors and ARBs might prevent collagen formation, but once crosslinking has occurred, they might not be able to reverse this process. This is a potential explanation for the demonstrated inability of ACE-inhibitors and ARBs to improve clinical outcome in HF patients with diastolic dysfunction.(7,9) Because of their novel and unique mode of action, we believe that AGE-breakers might become a novel therapy to improve clinical outcome

in patients with HF. Two small open-label studies with alagebrium have already shown promising effects in HF patients.(17,20)

Limitations

Two limitations of this study should be addressed. First, this is a relatively small study population, and a relatively large increase in peak VO_2 (15%) is needed to achieve a statistically significant result. Second, baseline aerobic capacity, which was measured with peak VO_2 in the present study, was 21.7 mL/min/kg, which is higher than expected. In addition, in our subgroup of patients with a LVEF ≤ 0.35, mean peak VO_2 was significantly lower than in patients with a LVEF > 0.35, but higher than in previous studies investigating the effect of interventions on exercise capacity.(29) The main reasons for this are; the selection of patients able to perform a reasonable cardiopulmonary exercise test, and the stable clinical condition of this very well-treated population. A peak VO_2 of 21.7 mL/min/kg is comparable to that reported in a similar study population by Lapu-Bula et al.,(30) in which patients with dilated cardiomyopathy had a mean peak VO_2 of 21 mL/min/kg. However, our sample size calculations were based on data from Mancini et al. in which peak VO_2 increased significantly from 11 ± 0.8 to 12.7 ± 2.8 mL/min/kg. As previously described, the peak VO_2 of our patients was better than anticipated, although peak VO_2 was still impaired. Therefore, our anticipated improvement of 15% might be optimistic, and it therefore remains to be seen whether the patient numbers are sufficient to detect meaningful changes.

Conclusion

Recent studies both in animals and humans have suggested that AGEs play a role in the development and progression of HF. AGE-breakers have been shown to improve left ventricular diastolic function in small uncontrolled studies.(17,31) A possible explanation could be that AGE-breakers improve the structural modifications that are caused by accumulation of AGEs. BENEFICIAL is the first prospective randomized placebo-controlled clinical study of an AGE-breaker in HF patients. If an improvement in exercise capacity and diastolic function is shown, this will be a major step towards a new treatment strategy in HF patients.

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Effects of alagebrium, an AGE breaker, on exercise tolerance and cardiac function in patients with chronic heart failure

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Abstract

Aims

Advanced glycation endproducts (AGEs) have been associated with the development and progression of chronic heart failure (HF). AGE-crosslink breakers might be of benefit in HF, but only small-scale and uncontrolled data are available. Our aim was to conduct a prospective, randomized, double-blind, placebo controlled study to examine the effects of the AGE-breaker alagebrium on exercise capacity and cardiac function in patients with HF.

Methods and Results

102 patients with HF (78% male, aged 62 ± 11 years), and a left ventricular ejection fraction ≤ 0.45, were randomized to either 200 mg alagebrium twice daily or placebo. After 36 weeks, the primary efficacy end-point peak VO_2 had changed with (mean ± SEM) -2.1 ± 0.5 ml/min/kg in alagebrium versus -0.5 ± 0.7 ml/min/kg in placebo treated patients ($p = 0.06$). No significant changes were observed in a number of secondary end-points, including diastolic function (mean E': $p = 0.32$; E/E': $p = 0.81$), systolic function (LVEF: $p = 0.43$), AGE accumulation (skin autofluorescence: $p = 0.42$), NT-proBNP ($p = 0.20$), NYHA functional class ($p = 0.73$), patient global assessment ($p = 0.32$), physicians global assessments ($p = 0.76$), and the Minnesota Living with Heart Failure Questionnaire score ($p = 0.38$). Overall alagebrium was reasonably well tolerated.

Conclusion

In the present proof-of-concept study, the AGE-breaker alagebrium did not improve exercise tolerance in patients with HF and systolic dysfunction and no changes were observed in a number of secondary endpoints. The present data therefore do not support earlier data which suggested a beneficial effect of alagebrium in HF.

Introduction

Chronic heart failure (HF) may occur in the presence of a preserved or depressed left ventricular ejection fraction (LVEF).(1) Both in patients with systolic and diastolic HF, diastolic dysfunction is present and related to symptoms.(2-5) Even though diastolic dysfunction is common and strongly related to symptoms, there is a poor understanding of its pathophysiology.(6) Several mechanisms underlying diastolic heart failure have been proposed(7-11). A potential explanation might be that diastolic dysfunction is related to structural modifications of the extracellular matrix by advanced glycation end-products (AGEs).(7,12) AGE accumulation occurs during life, but increased levels of AGEs are found in patients with diabetes, renal dysfunction, and hypertension. Accumulation of AGEs causes complex vascular as well as myocardial structural and functional changes via the interaction with AGE-receptors, leading to diastolic dysfunction.(7)

Advanced glycation end-product (AGE) crosslink breakers might be able to reverse structural changes that are related to diastolic dysfunction. Efficacy of AGE crosslink breakers in HF is supported by experimental work(13-16) and 2 small open label and uncontrolled clinical studies.(17,18) In 23 elderly patients with diastolic HF, 210 mg alagebrium twice daily given open-label for 16 weeks reduced left ventricular mass and improved diastolic function.(17) In another open-label study, 35 - 420 mg alagebrium improved diastolic function and left ventricular remodelling in 20 patients with systolic heart failure, although these results have only been published as an abstract.(18) Based on the pathophysiological mechanism, supported by small open-label and uncontrolled clinical studies, we initiated a prospective, randomized, double-blind, placebo controlled study on the effects of the AGE-breaker alagebrium on exercise capacity and cardiac function in patients with HF.

Methods

Patients and Study design

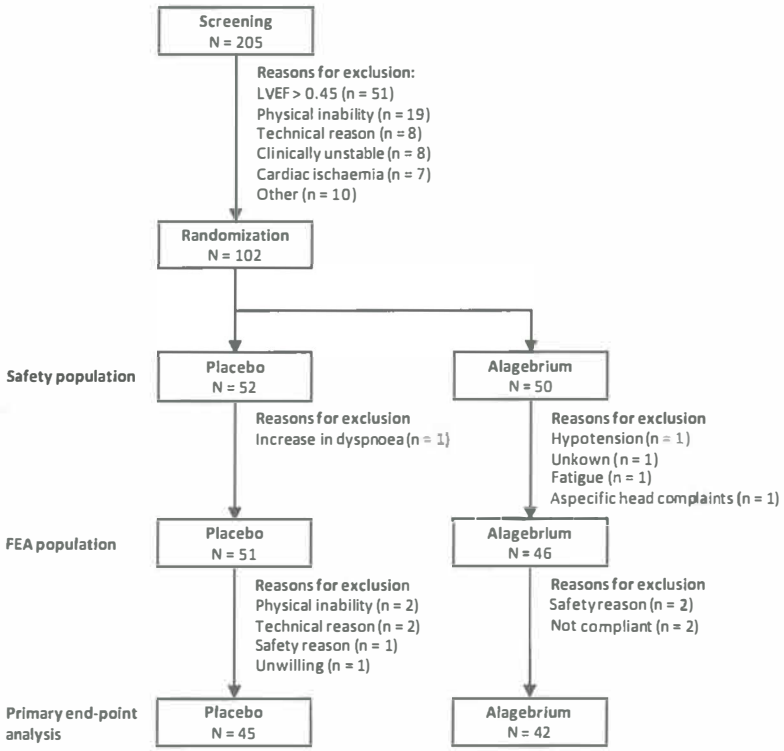
The BENEFICIAL (A double-blind, placebo-controlled, randomized trial evaluating the efficacy and safety of Alagebrium (ALT-711) in patients with chronic heart failure) trial is a prospective, randomized, double-blind, placebo-controlled, phase II study evaluating the efficacy and safety of alagebrium (ALT-711) in patients with CHF. The design and baseline characteristics of the BENEFICIAL trial have been published elsewhere.(19) Patients were recruited from the University Medical Center Groningen, and three other regional affiliated hospitals. In- and exclusion criteria are described in detail elsewhere.(19) Briefly, patients with NYHA II-IV stable HF for at least 3 months and a LVEF ≤ 0.45 were eligible for the study. Main exclusion criteria were the inability of patients to undergo exercise testing, cardiac resynchronisation therapy, pacemaker therapy, active and or treated malignancies within 12 months prior to inclusion, and clinically significant renal disturbance. A total of 205 patients were screened for the study (figure 1). One hundred and three patients were excluded. Main reasons for exclusion were LVEF > 0.45 (n = 51), not able to perform a peak VO_2 (n = 19), technically insufficient echocardiographic image quality (n = 8), not in clinical stable condition (n = 8), ischemia on cardiopulmonary aerobic capacity testing (n = 7), and other (n = 10). The remaining 102 patients with stable HF were randomized to either 200 mg alagebrium twice daily or placebo for a period of 36 weeks. Before randomization, a first rehearse cardiopulmonary aerobic capacity test was performed during a preceding visit to familiarise the patient with the procedures. Efficacy measurements, including cardiopulmonary aerobic capacity testing and echocardiography, were performed at baseline, and at 36 weeks. After randomization, safety visits were performed at 12 weeks intervals. In addition, extra safety visits were performed 2 weeks after randomization, and 4 weeks after study medication was stopped. Recruitment started November 2007 and was completed at the end of December 2008. The study was approved by the Medical Ethical Committee and all subjects gave written informed consent. (NCT00516646)

Randomization and Masking

Randomization was done via the use of the interactive voice response (IVR) system. Randomization data were kept strictly confidential until the time of unblinding. Drug codes were broken and made available for data analysis after the study was completed, and the data file was verified.

Figure 1 Patient inclusion and exclusion

This figure illustrates patient in-and exclusion. 205 patients were screened of whom 103 were excluded. from 102 randomized patients, 5 were not included in the full efficacy analysis (FEA) population. In 10 patients, we were unable to obtain primary end-point follow-up data, leaving 87 patients available for primary end-point efficacy analysis.



Cardiopulmonary aerobic capacity testing

Cardiopulmonary aerobic capacity testing was performed using a CareFusion, Masterscreen CPX (Houten, The Netherlands) according to a Modified Bruce protocol, which more gradually increases the workload than the Bruce protocol.(20) Oxygen uptake (VO_2), carbon dioxide production (VCO_2), and minute ventilation (VE) were measured using breath by breath gas analysis. Patients were encouraged to continue exercising until their VO_2-max was reached, they become uncomfortably symptomatic, or discontinuation was indicated for safety reasons.

Echocardiography

Echocardiography was performed by experienced cardiac technicians using a General Electric VIVID 7 system (Horton, Norway) with a 2.5-3.5-mHz probe. Measurements included left ventricular and atrial dimensions, peak early (E) and late (A) diastolic filling velocities, isovolumetric relaxation time (IVRT), and deceleration time (slope) of the early peak filling (DCT). Furthermore, using colour coded tissue Doppler imaging (ccTDI), early diastolic velocity (E') was measured on the lateral, and septal wall areas, and subsequently averaged. E/E' was calculated by dividing the peak early diastolic filling (E) by the mean E' measured using ccTDI. Systolic dysfunction was determined by calculating Simpson LVEF. When Simpson LVEF couldn't be determined, eyeballing LVEF was estimated. In 37 out of 97 patients simpson LVEF could not be determined and was estimated by eyeballing LVEF.

Skin autofluorescence

Tissue AGE accumulation was assessed using a validated skin autofluorescence (skin AF) reader (AGE-reader; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands), as described previously.(21,22) In short, the AGE reader illuminates a skin surface of approximately 2 cm^2, guarded against surrounding light, with an excitation light source between 300-420 nm (peak excitation ~ 370 nm). Light from the skin was measured with a spectrometer in the 420-600 nm range, using 200 µm glass fiber. The value of skin AF was calculated as the ratio of the light intensity in the 420-600 nm wavelength range, and the light intensity in the 300-420 nm wavelength range.

Endpoints

Primary endpoint of the study was the effect of alagebrium on aerobic capacity measured by cardiopulmonary exercise testing and determined as an average value of the two highest VO_2 values at peak performance expressed as ml/min/kg. Peak VO_2 was also expressed as ml/min/fat free mass as well as a percentage of predicted peak oxygen consumption. In addition to a first rehearse cardiopulmonary aerobic capacity test (screening), cardiopulmonary exercise testing was performed at baseline and after 36 weeks of treatment. Secondary endpoints were diastolic function expressed as change in mean E' and E/E', systolic function expressed as change LVEF, AGEs in tissue measured as change in skin AF, change in Minnesota Living with Heart Failure scores (MLHF), change in NYHA functional class, change in patient's and physician's global assessment scores, and change in levels of NT-proBNP.

Statistical considerations

Sample size calculation

The primary aim was to study the effect of alagebrium (ALT-711) on aerobic capacity. Based upon the data of Mancini et al.(23) the expectation was that an increase of at least 15% in peak VO_2 would be of clinical significance. To demonstrate an increase of 15% in aerobic capacity, with a power of at least 80% at a significance level of 0.05, 78 patients would be needed to study the primary end-point. With an expected drop-out of approximately 20%, a total number of 100 randomized patients would be needed.

Statistical analysis plan

Continuous variables were expressed as mean ± standard deviation (SD), mean ± standard error of the mean (SEM) or as median (25-75% interquartile range), where applicable. Nominal variables were expressed as n (%). Efficacy analyses were performed on the full efficacy analysis (FEA) population. Safety analyses were performed on all randomized patients (n = 102). The FEA population consisted of all randomized patients that continued medication until at least the 12-week visit. Patients that discontinued at or after the 12-week visit, and could be brought in for efficacy measurements within two weeks of stopping study medication, were included in the FEA population. Five patients were excluded from the FEA population because of early discontinuation, leaving 97 patients available for efficacy analyses (figure 1). The FEA population was used to calculate baseline characteristics (table 1). Baseline characteristics were analysed for difference over treatment groups by using ANOVA or Mann-Whitney-U test where applicable for continuous variables and by Chi-square for nominal variables. Primary and secondary efficacy analyses on group differences were performed by using ANOVA for normally distributed variables or Mann-Whitney-U test for skewed distributed variables. For qualitative parameters, group differences were evaluated using a Chi-square test. For subgroup analyses P for interaction was calculated using general linear modelling with ANOVA. No attempt was made to replace missing values. All statistical tests were two-sided. A p-value of less than 0.05 was considered to be statistically significant. Data were analysed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Table 1 Baseline characteristics of 97 systolic chronic heart failure patients randomized to alagebrium or placebo

Variable	Placebo (N = 51)	Alagebrium (N = 46)	p-value
Age(years)	59 ± 11	64 ± 11	0.03
Sex (male), n (%)	39 (77)	37 (80)	0.64
Diabetes Mellitus, n (%)	7 (14)	10 (22)	0.30
Race (Caucasian), n (%)	50 (98)	45 (98)	0.94
History of hypertension, n (%)	15 (29)	17 (37)	0.43
History of hypercholesterolemia, n (%)	32 (63)	30 (65)	0.80
Smoking, n (%)	12 (24)	11 (24)	0.97
Body mass index (kg/m^2)	28 ± 4	28 ± 4	1.00
Systolic blood pressure (mmHg)	116 ± 16	115 ± 15	0.71
Diastolic blood pressure (mmHg)	74 ± 9	71± 9	0.05
Heart rate (bpm)	71 ± 15	66 ± 11	0.06
Etiology of HF			0.94
Ischemic, n(%)	33 (65)	35 (76)	
Non-ischemic, n (%)	18 (35)	11 (24)	
Idiopathic	15 (29)	7 (15)	
Other	3 (6)	4 (9)	
NYHA functional class, n (%)			0.97
II	32 (63)	30 (65)	
III	18 (35)	14 (31)	
IV	1 (2)	2 (4)	
Laboratory assessments			
eGFR (MDRD) ($mL/min/1.73\ m^2$)	79 ± 22	81 ± 20	0.60
NT-proBNP (ng/L)	267 (93-631)	465 (204–964)	0.04
HbA1c (%)	5.7 (5.5-6.0)	5.7 (5.6-6.2)	0.47
Total cholesterol (mmol/l)	4.4 ± 1.0	4.3 ± 1.2	0.84
Skin AF (a.u.)	2.2 ± 0.5	2.4 ± 0.7	0.20
MLHF questionnaire			
Total score	18 ± 14	23 ± 19	0.14
Physical sub score	9 ± 6	11 ± 9	0.32
Echocardiography			
E/A	0.9 (0.7-1.1)	0.9 (0.7-1.3)	0.63
Dct (ms)	212 ± 58	217 ± 57	0.71
IVRT (ms)	104 ± 26	99 ± 20	0.41
E/E'	12.1 (10.1-15.5)	14.3 (9.5-21.3)	0.61
Mean E' (cm/s)	5.1 ± 1.8	5.1 ± 2.1	0.95

Table 1 Continued

Variable	Placebo (N = 51)	Alagebrium (N = 46)	p-value
LVEF	0.33 ± 0.09	0.33 ± 0.09	0.99
LVEDD (mm)	58.7 ± 8.2	58.5 ± 7.9	0.92
LVESD (mm)	48.0 ± 9.7	47.0 ± 10.2	0.63
VO_2 max exercise test			
Peak VO_2 (ml/min/kg)	22.5 ± 6.4	21.1 ± 5.8	0.28
Peak VO_2 (ml/min/kg fat free mass)	33.3 ± 9.2	31.0 ± 7.6	0.18
Peak VO_2 (% predicted VO_2 max)	84.4 ± 26.0	86.0 ± 24.6	0.75
Medication use, n (%)			
ACEi/ARB	48 (94)	43 (94)	0.90
ß-Blockers	48 (94)	44 (96)	0.73
Diuretics	30 (59)	27 (59)	0.99
Aldosteron antagonists	14 (27)	15 (30)	0.83

Bpm, beats per minute; HF, heart failure; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; NT-proBNP, N-terminal pro brain natriuretic peptide; HbA1c, hemoglobin A1c; skin AF, skin autofluorescence; MLHF, Minnesota Living with Heart Failure; DCT, deceleration time; IVRT, isovolumetric relaxation time; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker

Results

Baseline characteristics of the FEA population stratified according to treatment allocation are depicted in table 1. Mean age was 62 ± 11 years, 78% was male, 18% was diabetic and mean systolic blood pressure was 115 ± 15 mmHg. The study population was generally well balanced, except for a slightly higher age, lower diastolic blood pressure, and higher baseline NT-proBNP levels in the alagebrium group.

Primary end-point efficacy analysis

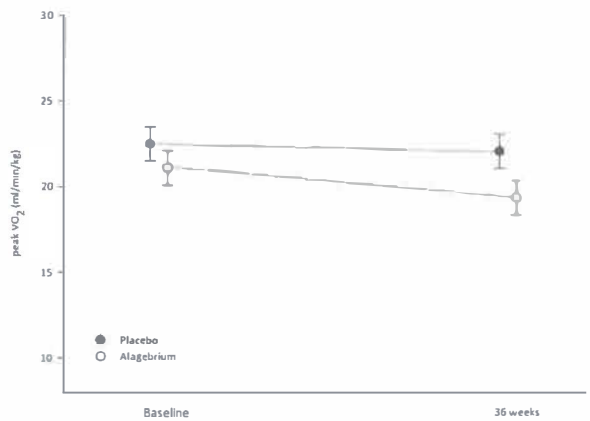
Results of primary end-point efficacy analysis are summarised in table 2 and figure 2. For various reasons, provided in figure 1, 10 patients were unable to perform a cardiopulmonary exercise test at 36 weeks, leaving 87 patients available for primary end-point efficacy analysis. Aerobic exercise capacity measured as peak VO_2 (ml/min/kg) changed with -2.1 ± 0.5 ml/min/kg in alagebrium versus -0.5 ± 0.7 ml/min/kg in placebo treated patients (p = 0.06 for change between groups). This effect was of similar magnitude when peak VO_2 was expressed in ml/min/kg fat free mass (p=0.04) and as percentage of predicted peak VO_2 (p = 0.07). No significant differences were observed for respiratory quotient (p = 0.37), anaerobic threshold (p = 0.69), and T ½ peak VO_2 (p = 0.15).

Table 2 Primary end-point efficacy analysis

Variable (mean ± SEM)	Placebo N=45	Alagebrium N=42	P-value
Peak VO_2 (ml/min/kg)			
Baseline	22.5 ± 1.0	21.4 ± 0.9	
Follow-up	22.1 ± 0.9	19.4 ± 0.8	
Difference	-0.5 ± 0.7	-2.1 ± 0.5	0.06
Peak VO_2 (ml/min/kg fat free mass)			
Baseline	33.3 ± 1.4	31.2 ± 1.2	
Follow-up	33.1 ± 1.4	28.3 ± 1.1	
Difference	-0.2 ± 1.0	-2.9 ± 0.7	0.04
Peak VO_2 (% predicted)			
Baseline	85.4 ± 3.9	87.2 ± 3.9	
Follow-up	83.7 ± 3.2	80.0 ± 3.7	
Difference	-1.7 ± 2.4	-7.3 ± 1.9	0.07
Respiratory Quotient			
Baseline	1.06 ± 0.02	1.08 ± 0.02	
Follow-up	1.08 ± 0.02	1.08 ± 0.02	
Difference	0.02 ± 0.02	0.00 ± 0.01	0.37
Anaerobic threshold (ml/min/kg)			
Baseline	13.2 ± 0.5	12.8 ± 0.4	
Follow-up	13.8 ± 0.5	13.1 ± 0.5	
Difference	0.6 ± 0.5	0.3 ± 0.4	0.69
T½ peak VO_2 (seconds)			
Baseline	94 ± 6	98 ± 6	
Follow-up	94 ± 5	108 ± 6	
Difference	0 ± 5	11 ± 6	0.15

Figure 2 Changes in peak VO_2 stratified according to treatment group

This figure illustrates the results of the primary end-point efficacy analysis. Depicted are mean peak VO_2 values at baseline as well as the 36 weeks visit. Error bars indicate SEMs. Aerobic exercise capacity measured as peak VO_2 (ml/min/kg) changed from 22.5 ± 1.0 to 22.1 ± 1.0 ml/min/kg in placebo and from 21.4 ± 0.9 to 19.4 ± 0.8 ml/min/kg in alagebrium treated patients (p = 0.06 for change between groups).



Secondary end-point efficacy analysis

Results of secondary end-point efficacy analysis are summarised in table 3. No significant differences were observed in changes in mean E' (p = 0.32), E/E' (p = 0.81), LVEF (p = 0.43), skin AF (p = 0.42), NT-proBNP (p = 0.20), NYHA functional class (p = 0.73), patient global assessment (p = 0.32), physicians global assessment (p = 0.76) and MLHF score (p = 0.38). Although left ventricular end-diastolic and systolic diameter (LVEDD and LVESD) were not defined as a pre-specified secondary end-point they were evaluated to establish whether the use of alagebrium was associated with changes in left ventricular dimensions. No end diastolic dilatation was observed in the alagebrium treated patients (p-value for within group analysis: p = 0.35). LVEDD changed from (mean ± SEM) 58.7 ± 1.2 mm to 56.8 ± 1.1 mm in placebo and 58.7 ± 1.3 mm to 59.4 ± 1.3 mm in alagebrium treated patients (p-value for between group analysis: p = 0.01). LVESD changed from (mean ± SEM) 48.0 ± 1.4 mm to 46.3 ± 1.4 mm in placebo and 47.4 ± 1.7 mm to 48.6 ± 1.7 mm in alagebrium treated patients (p-value for between group analysis: p = 0.03; p-value for within group analysis: p = 0.26).

Subgroup analyses

Pre-specified subgroup analyses were performed for subgroups defined by diabetes, age, etiology of CHF, NYHA functional class, gender, peak VO_2, LVEF, mean E', E/E', NT-proBNP, and skin AF. Results of subgroup analyses are summarised in figure 3. No significant interaction was observed.

Safety analysis

Safety analyses were performed on all randomized patients (n = 102). In total nine patients discontinued treatment, of which 6 were using alagebrium. Reasons for discontinuation in alagebrium treated patients were symptomatic hypotension (n = 1), and unwilling to further participate without clear reason (n = 5). Reasons for discontinuation in placebo treated patients were ventricular arrhythmias (n = 1), and unwilling to further participate without clear reason (n = 2). Serious adverse events (SAEs) and adverse events (AEs) are summarised in table 4. In total 41 SAEs were reported of which 20 in alagebrium treated patients. One patient in the alagebrium group died as a consequence of lung cancer. Lung cancer was diagnosed shortly after patient completed the study protocol. No differences in incidence rates were observed in death and/or hospitalisations. In total 319 AEs were reported of which 175 in alagebrium treated patients. Alagebrium treated patients more often had dyspepsia (p = 0.04), and reported significantly less nasopharyngitis (p=0.04), and musculoskeletal discomfort (p = 0.02).

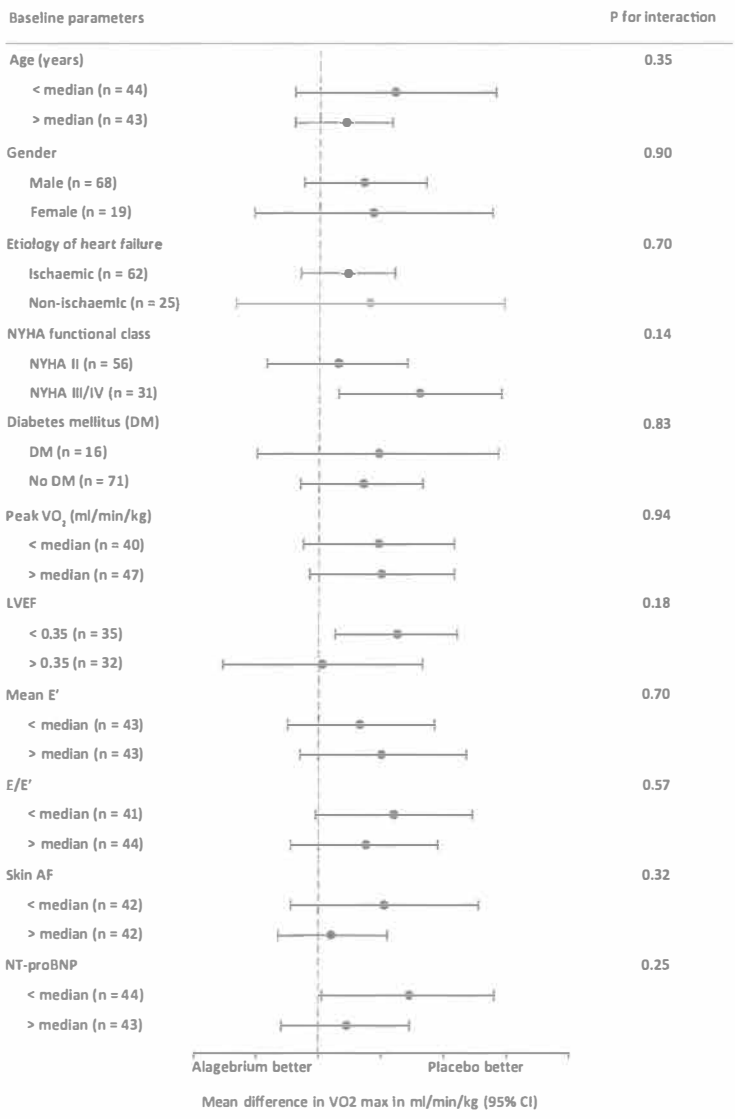
Table 3 Secondary end-point efficacy analysis

Variable			Placebo N=51	Alagebrium N=46	p-value
Mean E' (cm/s)	Baseline		5.2 ± 0.3	5.1 ± 0.3	
	Follow-up		5.2 ± 0.3	4.7 ± 0.4	
	Difference		0.0 ± 0.2	-0.3 ± 0.3	0.32
E/E'	Baseline		13.6 ± 1.0	17.1± 1.8	
	Follow-up		12.9 ± 1.2	16.8± 1.6	
	Difference		-0.7 ± 1.0	-0.2 ± 1.7	0.81
LVEF	Baseline		0.33 ± 0.01	0.33 ± 0.01	
	Follow-up		0.34 ± 0.02	0.32 ± 0.02	
	Difference		0.01 ± 0.01	-0.01 ± 0.01	0.43
Skin AF (a.u)	Baseline		2.2 ± 0.1	2.3 ± 0.1	
	Follow-up		2.1 ± 0.1	2.2 ± 0.1	
	Difference		-0.1 ± 0.1	-0.2 ± 0.1	0.42
NT-proBNP (ng/L)	Baseline		267 (93 - 631)	465 (204 - 964)	
	Follow-up		246 (101 - 800)	407 (198 - 936)	
	Difference		8 (-60 - 159)	-14 (-220 - 97)	0.20
NYHA functional class (%)	Baseline				0.73
		I/II	32 (63)	30 (65)	
		III/IV	19 (37)	16 (35)	
	Follow-up				
		I/II	40 (82)	33 (75)	
		III/IV	9 (18)	11 (25)	
Patient Global Assessment (cumulative score)			0.7 ± 0.2	0.4 ± 0.2	0.32
Physicians Global Assessment (cumulative score)			0.5 ± 0.2	0.5 ± 0.2	0.76
MLHF Questionaire (total score)	Baseline		18 ± 2	21 ± 3	
	Follow-up		18 ± 2	25 ± 3	
	Difference		1 ± 2	3 ± 2	0.38
MLHF Questionnaire (physical sub score)	Baseline		9 ± 1	10 ± 1	
	Follow-up		9 ± 1	11 ± 1	
	Difference		0 ± 1	1 ± 1	0.21

Continuous variables were expressed as mean ± SEM or as median (25 - 75% interquartile range), where applicable. Nominal variables are expressed as n (%). Patient and physicians global assessment were scored per follow-up visit as much worse (-2), worse (-1), stable (0), better (1), much better (2). Cumulative score was used for analyses. Abbreviations NYHA New York Heart Association; skin AF, skin autofluorescence; NT-proBNP, N-terminal pro brain natriuretic peptide. MLHF Minnesota Living with Heart Failure; LVEF, left ventricular ejection fraction.

Figure 3 Pre-specified subgroup analysis

This Figure illustrates the results of the pre-specified subgroup analysis. Depicted are means ± 95% confidence intervals (CI). Specific subgroup means were calculated by subtracting mean VO_2-max (ml/min/kg) in alagebrium treated patients from mean VO_2-max in placebo treated patients. P for interaction was calculated using general modelling with ANOVA.



NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; Skin AF, skin autofluorescence; NT-proBNP, N-terminal pro brain natriuretic peptide

Table 4 Safety results

Variable	Placebo (n=52)	Alagebrium (n=50)	p-value
Serious Adverse Events			
Death	0 (0)	1 (2)	0.31
All-cause hospitalizations	20 (38)	21 (42)	0.72
Cardiovascular hospitalizations	12 (23)	10 (20)	0.71
ICD implantations/replacements	3 (6)	4 (8)	0.66
Other	5 (10)	7(14)	0.49
Adverse Events			
Cardiacvascular			
Rhythm disturbances	7 (13)	14 (28)	0.07
Fatigue	11 (21)	13 (26)	0.57
Dyspnoea	7 (13)	11 (22)	0.26
Blood pressure fluctuation	4 (8)	9 (18)	0.12
Cardiac failure	3 (6)	9 (18)	0.06
Chest pain	1 (2)	5 (10)	0.08
Gastrointestinal			
Gastrointestinal symptoms	7 (13)	13 (26)	0.11
Dyspepsia	0 (0)	4 (8)	0.04
Nausea	1 (2)	4 (8)	0.16
Musculoskeletal			
Musculoskeletal discomfort	13 (25)	4 (8)	0.02
Arthralgia	1 (2)	3 (6)	0.29
Gout	5 (10)	3 (6)	0.50
Respiratory			
Nasopharyngitis	16 (31)	7 (14)	0.04
Exacerbation COPD	2 (4)	5 (10)	0.22
Urogenital			
Renal impairment	2 (4)	4(8)	0.37
Urinary tract infection	4 (8)	6(12)	0.47
Miscellaneous			
Dizziness	4 (8)	8 (16)	0.19
Malaise	3 (6)	5 (10)	0.43
Non-cardiac chest pain	5 (10)	3 (6)	0.50
C-reactive protein increased	0 (0)	3 (6)	0.07
Weight fluctuation	5 (10)	2 (4)	0.26
Glucose disregulation	6 (12)	1 (2)	0.06
Other	37 (71)	43 (86)	

Discussion

The present study is the first report of a placebo-controlled study on the effects of an AGE-crosslink breaker in HF. Despite several pathophysiological considerations and limited clinical data suggesting beneficial effects, our data do not support the use of AGE-breakers in HF patients, as there was no improvement of exercise capacity or any other clinical marker.

AGEs have been proposed as an important mechanism in heart failure.(7,12) AGEs are carbohydrate and lipid dependent modifications of protein, formed by oxidative or non-oxidative reactions. They affect the physiological properties of proteins in the extracellular matrix, such as charge, hydrophobicity, turnover, and elasticity. The latter is caused by an important mechanism of AGEs, the formation of collagen crosslinks, which results in increased myocardial stiffness. Second to that, AGEs cause complex vascular as well as myocardial structural and functional changes via the interaction with AGE-receptors. The deleterious effect of AGEs can be targeted in several ways, of which the use of AGE crosslink breakers has shown to be most promising.

The AGE crosslink breaker alagebrium has been examined for several indications, including hypertension, peripheral vascular disease, and HF both in experimental settings, as well as clinical studies. Alagebrium improved endothelial function(24), arterial compliance(25), and diastolic function.(17,18) In 23 elderly patients with diastolic HF, 210 mg alagebrium twice daily given open-label for 16 weeks reduced left ventricular mass and improved diastolic function. However, patients participating in this trial were patients with diastolic HF and LVEF above 0.50, while we included patients with ejection fraction ≤ 0.45. Of note, this study was open label and did not have a control group. In another open-label and uncontrolled study, 35-420 mg alagebrium improved diastolic function and left ventricular remodelling in 20 patients with systolic HF, although these results have only been published as an abstract. (18)

Despite a strong background and rationale, the AGE crosslink breaker alagebrium did not improve exercise capacity or cardiac function in the current study. The selection of our patient population could have influenced our findings. Although patient with systolic dysfunction frequently have diastolic dysfunction as well, we did not specifically select patients with diastolic dysfunction, in whom the largest effect of alagebrium could be expected. Additionally, we observed a small trend towards a more pronounced negative treatment effect of alagebrium in patients with low LVEFs in subgroup analysis. Second, tissue AGE levels were relatively low in our population, with only very few diabetic patients, and therefore the contribution of AGE-crosslinks to their impaired exercise capacity might

have been neglectable. Third, the duration of treatment might have been too short to induce clinical impact in this population. Finally, despite a scientific rationale that AGEs play a role in the pathophysiology of HF, AGE-crosslink breakers might not be the proper therapy to interfere with this process.

After 36 weeks of treatment, LVEDD remained unchanged in the alagebrium group, while it decreased in the placebo group. Similar findings were observed for LVESD. From a pathophysiological perspective one may hypothesise that AGE breaking therapy reduces AGE crosslinks, thus increasing left ventricular compliance and inducing left ventricular dilatation. Evidence does exist that alagebrium therapy is associated with aortic dilatation in elderly hypertensive dogs, but left ventricular properties in this study remained unaffected. (26) Also, in the aforementioned smaller clinical studies(17,18) no increase was observed in left ventricular end-diastolic volume.

We performed a pre-specified subgroup analysis to investigate whether the effects of AGE-breaking therapy would be more pronounced in certain sub-groups. We expected to find a more pronounced effect of AGE-breaking therapy in the older, diabetic patients with worse diastolic function and high baseline AGE levels. However, no such significant interactions were observed. A possible explanation for this unexpected result could be the low amount of patients with these specific features, especially with respect to the low amount of patients with diabetes.

AGE accumulation was measured by skin autofluorescence (skin AF). No treatment effect of alagebrium was found on skin AF. One can question whether to expect a treatment effect, since it is unknown how AGE crosslink breakers influence circulating and tissue AGE levels.

Generally, alagebrium appeared to be safe and well tolerated. We found no increases in the incidence of death and/or hospitalisations, and the use of alagebrium was associated only with some mild gastrointestinal discomfort. Also, some trends ($p < 0.10$) were observed towards more dizziness, rhythm disturbances, cardiac failure, chest pain, and increase in CRP. The small decrease in exercise tolerance found in this study is potentially of concern. Although we could not fully explain its causes, it could be a chance finding.

Limitations

Our study population consisted of well treated HF patients with relatively low NT-proBNP levels at baseline, probably related to the selection of patients that were able to undergo exercise testing. Therefore, severely symptomatic patients, very elderly patients, and those with serious co-morbidities, limiting their exercise capacity were less likely to be included in this study. Results can therefore not be readily applied to more severely symptomatic HF patients. Also, although we feel that our study was adequately powered, it remains a phase II study with a small number of patients. Finally, we selected a group of heart failure patients with reduced ejection fraction, and the results might have been different in patients with HF with a preserved ejection fraction (HF-PEF). However, using a mean tissue Doppler velocity (E') of < 8 cm/sec, 85% of our patients had diastolic dysfunction.

Conclusions

In the present proof-of-concept study, the AGE-breaker alagebrium did not improve exercise tolerance in patients with HF and systolic dysfunction, and no changes were observed in a number of secondary endpoints. The present data therefore do not support earlier data which suggested a beneficial effect of alagebrium in HF.

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Effect of the AGE-breaker alagebrium on plasma and tissue advanced glycation end-products and sRAGE

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Abstract

Introduction

Advanced glycation end-products (AGEs) are crystallized proteins that accumulate during ageing, but are particularly increased in patients with diabetes, renal and/or heart failure (HF). The AGE crosslink breaker alagebrium cleaves AGE-derived protein. However, the effects of an AGE-breaker on soluble receptor of AGE (RAGE), plasma and tissue AGE levels remain to be established.

Methods

In 96 patients, included in a randomized, double-blind, placebo controlled study, sRAGE, plasma and tissue AGE levels were measured before start of treatment with alagebrium or placebo and after 36 weeks of treatment. N^{ϵ}-(carboxymethyl)lysine (CML) was determined by liquid chromatography mass spectrometry and pentosidine by high performance liquid chromatography. sRAGE was determined by enzyme-linked immunosorbent assay (ELISA).

Results

Mean age was 61 ± 11 years, 80% were male, 17% were diabetic. Mean CML was 60 ± 14 nmol/mmol lysine, median pentosidine was 0.58 (0.46 - 0.72) nmol/mmol lysine and median sRAGE was 1012 (637 - 1705) pg/mL. No significant changes on AGE levels were observed between baseline and after 36 weeks of treatment for levels of plasma AGE CML and pentosidine, tissue AGE and sRAGE (respectively $p = 0.42$, $p = 0.73$, $p = 0.93$ and $p = 0.57$).

Conclusion

This randomized, placebo controlled study showed that the AGE-breaker alagebrium did not change levels of CML, pentosidine, tissue AGE or sRAGE in systolic HF patients. These findings support the absence of an effect of alagebrium on exercise capacity and cardiac function in these patients.

Introduction

Recently, it was suggested that advanced glycation end-products (AGEs) play a role in the pathogenesis of heart failure (HF).(1,2) AGEs are molecules formed during a non-enzymatic reaction between protein and sugar residues, called a Maillard reaction.(1) AGEs accumulate in the body during ageing, and are, next to that, increased in patients with chronic systolic or diastolic HF, diabetes and/or renal dysfunction.(1,2) Furthermore, tissue AGE levels are associated with diastolic dysfunction (2), which might be caused by crosslinking capabilities of AGEs and/or the binding capacity of AGEs to the receptor of AGE (RAGE).(1-4) Upon interaction of AGE with RAGE cardiovascular dysfunction occurs. However, RAGE has a secretory isoform, soluble RAGE (sRAGE), which has been proposed to interfere with AGEs' ability to bind and activate RAGE.(5-8)

Elevated AGE levels are associated with a reduced survival rate in, among others, HF patients, and may therefore be target for intervention. AGE-crosslink breakers might be able to reverse AGE-induced crosslinks. By breaking these crosslinks, structural changes that are related to diastolic dysfunction might be reversed.(9) In a small, open label, uncontrolled clinical study, alagebrium improved diastolic function and reduced left ventricular mass in 23 patients with diastolic HF.(10) However, in a randomized, placebo controlled study (BENEFICIAL), alagebrium did not improve exercise capacity and diastolic function in 102 patients with systolic HF.(11) Experimental studies showed that alagebrium reduced tissue AGE accumulation and reversed some of the cardiovascular complications resulting from ageing, hypertension and diabetes.(12-16) However, in humans, the effect of alagebrium on AGEs remain to be established.

We therefore studied the effects of alagebrium on levels of plasma AGEs, tissue AGE and sRAGE in a sub-study of the BENEFICIAL.

Methods

Patients and study design

The present study analyzed data of all patients that were randomized in a prospective, double-blind, placebo-controlled trial evaluating the efficacy and safety of alagebrium (200 mg twice daily) in patients with chronic HF (BENEFICIAL, NCT00516646). The study was approved by the Medical Ethical Committee and all subjects included gave written informed consent. The study design and baseline characteristics of the study are published elsewhere.(4) The main results were recently presented.(11) In brief, 102 patients with NYHA II-IV stable HF for at least 3 months and left ventricular ejection fraction (LVEF) ≤ 0.45 were randomized to either alagebrium 200 mg b.i.d. or placebo for a period of 36 weeks.

Skin autofluorescence (skin AF)

Tissue AGE accumulation was assessed using a validated skin AF reader (advanced glycation end-products reader; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands) described previously.(17,18) In short, the AGE-reader illuminates a skin surface of approximately 2 cm^2, guarded against surrounding light, with an excitation light source between 300-420 nm (peak excitation ~ 370 nm). Light from the skin is measured with a spectrometer in the 420-600 nm range, using 200 µm glass fibers. The value of skin AF is calculated as the ratio of the light intensity in the 420-600 nm wavelength range, and the light intensity in the 300-420 nm wavelength range. Skin AF was measured at the volar side of the lower arm at approximately 10 to 15 cm below the elbow fold. The measurement was performed three times at healthy skin site (i.e., without visible vessels, scars, or other skin abnormalities) and an average was calculated.

Determination of N^{ϵ}-(carboxymethyl)lysine

N^{ϵ}-(carboxymethyl)lysine (CML) was determined by stable-isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS).(19) In short, CML was liberated from plasma proteins by acid hydrolysis after addition of deuterated CML as internal standards. Chromatographic separation was performed by gradient-elution reversed-phase chromatography with a mobile phase containing 5 mmol/L nonafluoropentanoic acid as ion-pairing agent. Mass transitions of 205.1 → 84.1 CML, and 209.1 → 88.1 and 223.1 → 88.1 for the respective internal standards was monitored in positive-ion mode. CML was separated by baseline resolution with a total analysis time of 21 min. Within-day and between-day coefficients of variation were < 4.4% and < 3.2%.

Determination of pentosidine levels

Pentosidine levels were measured by high performance liquid chromatography (HPLC).(20) Plasma proteins were hydrolysed in 6 N HCL. Detection of pentosidine is done based on its own fluorescence characteristics using fluorimetric detection (E_x = 325 nm, E_m = 385 nm). Separation is done, with a run-to-run time of 30 min, on a C18 Allspehere ODS-II column with a citric acid acetonitrile buffer. This detection enables sensitive and specific determination of protein bound pentosidine in plasma with a detection limit of 2.2 nmol/l or 0.02 pmol/mg.

Determination of soluble receptor for advanced glycation end products

Soluble receptor for advanced glycation end products (sRAGE) was measured using commercially available enzyme linked immunosorbent (ELISA) techniques, according to manufacturer's instructions (R&D Systems, Minneapolis, USA). This method has an inter-and intra-assay variation of less then 5%.

Statistical analysis

Variables which are normally distributed are expressed as mean ± SD, whereas non-normally distributed variables are expressed as median (25% to 75% interquartile range), and nominal parameters are expressed as n (%). Differences in characteristics between patient groups were analyzed within each group using a t-test when the parameter is normally distributed, or a Mann-Whitney U test when normality is not met. Correlation coefficients were calculated using Pearson correlation coefficients. Variables were considered in the multivariate models when a p-value < 0.1 was obtained in univariable regression. Variables which not retained significance in the multivariable regression analysis were subsequently removed from the model (backward selection). To test whether the model is appropriate and whether assumptions for linear regression are met, the model was tested for colinearity, interaction terms, and lack-of-fit analysis with variance. Residuals were tested for normality of distribution. Analyses were performed with SPSS 18.0.3 (SPSS Inc., Chicago, IL, USA). All statistical tests were two sided and a p-value < 0.05 was considered statistically significant.

Table 1 Baseline characteristics

Paramaters	Total population (n=96)	Placebo N=48	Alagebrium N=48	p-value
Age(years)	61 ± 11	59 ± 11	64 ± 12	0.05
Sex (male), n (%)	77 (80)	38 (79)	39 (81)	0.80
Diabetes, n (%)	16 (17)	6 (13)	10 (21)	0.42
Race (caucasian), n (%)	94 (98)	47 (98)	47 (98)	1.00
History of hypertension, n (%)	31 (32)	14 (29)	17 (35)	0.51
History of hypercholesterolemia, n (%)	62 (65)	32 (67)	30 (63)	0.67
Smoking, n (%)	23 (24)	12 (25)	11 (23)	0.81
Body mass index (kg/m^2)	28 ± 4	28 ± 4	28 ± 5	0.95
Diastolic blood pressure (mmHg)	72 ± 9	74 ± 9	70 ± 9	0.05
Systolic blood pressure (mmHg)	115 ± 15	116 ± 16	114 ± 15	0.47
Heart rate (b.p.m.)	69 ± 15	71 ± 15	68 ± 14	0.09
Aetiology of heart failure, n (%)				0.38
Ischaemic	66 (69)	31 (65)	35 (73)	
Non-ischaemic	30 (31)	17 (35)	13 (27)	
NYHA functional class, n (%)				0.83
II	61 (64)	30 (62)	31 (65)	
III/IV	35 (36)	18 (38)	17 (35)	
Laboratory assessments				
eGFR (MDRD) (mL/min/1.73 m^2)	80±21	79 ± 21	81 ± 20	0.74
NT-proBNP (ng/L)	394 (155 - 819)	308 (93 - 643)	444 (204 - 917)	0.07
HbA1c (%)	5.7 (5.5 - 6.2)	5.7 (0.9 - 3.1)	5.7 (5.6 - 6.2)	0.37
HsCRP	1.6 (0.9 - 3.8)	1.5 (0.9 - 3.1)	2.1 (0.8 - 4.1)	0.36
Total cholesterol (mmol/l)	4.4 ± 1.1	4.4 ± 1.0	4.4 ± 1.2	0.88
CML (nmol/mmol Lysine)	60 ± 14	59 ± 14	60 ± 14	0.87
CML (µmol/L)	2.7 ± 0.9	2.7 ± 0.9	2.7 ± 0.9	0.93
Pentosidine (nmol/ mmol Lysine)	0.58 (0.46 - 0.72)	0.58 (0.45 - 0.70)	0.59 (0.47 - 0.74)	0.48
Pentosidine (µmol/L)	0.03 (0.02 - 0.03)	0.03 (0.02 - 0.03)	0.03 (0.02 - 0.04)	0.67
sRAGE (pg/mL)	1012 (637 - 1705)	895 (647 - 1813)	1127 (636 - 1665)	0.86
Skin AF (a.u.)	2.3 ± 0.6	2.2 ± 0.5	2.3 ± 0.7	0.35
Medication use, n (%)				
ACE-i/ARB	91 (95)	45 (94)	46 (96)	0.65
ß-Blockers	90 (94)	46 (96)	44 (92)	0.40
Diuretics	58 (60)	28 (58)	30 (63)	0.68

NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; NT-proBNP, N-terminal-pro brain natriuretic peptide; HbA1c, haemoglobin A1c; HsCRP, high sensitive C-reactive protein; CML, N$^{\varepsilon}$-(carboxymethyl)lysine; sRAGE, soluble receptor of advanced glycation end-products; skin AF, skin autofluorescence; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker;

Results

Patient characteristics

A total of 102 patients were randomized for the main study. In 6 patients plasma was not obtained, so 96 patients were included in this sub-study. Baseline characteristics of the study population are depicted in table 1. Mean age was 61 ± 11 years, 77 (80%) were male, 17% was diabetic and mean systolic blood pressure was 115 ± 15 mmHg. Mean eGFR was 80 ± 21 mL/min/1.73 m^2, mean CML was 60 ± 14 nmol/mmol lysine, median pentosidine was 0.58 (0.46 - 0.72) nmol/mmol lysine and median sRAGE was 1012 (637 - 1705) pg/mL. Patients were well treated, 95% of the patients used an ACE-inhibitor or an ARB and 94% a β-blocker. Baseline characteristics were generally well balanced between the groups, except for age and diastolic blood pressure (table 1).

Changes between alagebrium and placebo after 36 weeks of treatment

Baseline and 36 week levels of plasma CML, pentosidine, sRAGE, and tissue AGE in alagebrium and placebo treated patients are presented in table 2. Overall, mean concentrations of CML, pentosidine, sRAGE, and tissue AGE remained unchanged during the 36 weeks follow-up time. Also, there were no significant differences between alagebrium and placebo groups.

Table 2 Change in plasma AGEs, tissue AGEs and sRAGE

	Placebo (N = 48)			Alagebrium (N = 48)			p-value
Variables	Baseline	36 weeks	Delta	Baseline	Placebo	Delta	
CML (nmol/mmol lysine)	59 ± 14	60 ± 12	0.46 ± 8.8	60 ± 14	61 ± 14	-0.65 ± 8.1	0.42*
Pentosidine (nmol/mmol lysine)	0.61 ± 0.2	0.59 ± 0.2	0.03 ± 0.2	0.65 ± 0.3	0.63 ± 0.2	0.04 ± 0.2	0.73*
Skin AF (a.u.)	2.2 ± 0.5	2.1 ± 0.6	-0.10 ± 0.5	2.3 ± 0.7	2.2 ± 0.6	-0.17 ± 0.5	0.93*
log sRAGE (pg/mL)	3.0 ± 0.3	3.0 ± 0.3	-0.04 ± 0.2	3.0 ± 0.3	3.0 ± 0.3	0.00 ± 0.2	0.57*

CML, N$^{\varepsilon}$-(carboxymethyl)lysine; skin AF, skin autofluorescence; sRAGE, soluble receptor of advanced glycation end-product

*, corrected for baseline values

Correlation

CML was significantly correlated with pentosidine ($r = 0.68$; $p < 0.001$), age ($r = 0.26$; $p = 0.01$) and eGFR ($r = -0.42$; $p < 0.001$). sRAGE was only significantly correlated with HbA1c ($r = 0.29$; $p = 0.01$). Finally skin AF was significantly correlated with age ($r = 0.43$; $p < 0.001$) and HbA1c ($r = 0.24$; $p = 0.02$) (table 3).

Linear regression plasma AGEs and sRAGE

CML was in univariable regression analysis associated with age, hypertension, body mass index, eGFR and NT-proBNP ($p = 0.01$, $p = 0.09$, $p = 0.002$, $p < 0.001$, $p = 0.001$; respectively). Multivariable linear regression analysis showed that CML was independently associated with body mass index, eGFR and NT-proBNP ($p = 0.001$, $p < 0.001$, $p = 0.001$; respectively). Univariable analysis showed that pentosidine was associated with hypertension, diastolic blood pressure, eGFR, NT-proBNP and skin AF ($p = 0.04$, $p = 0.01$, $p = 0.06$, $p < 0.001$, $p = 0.09$; respectively). In multivariable linear regression analysis pentosidine was associated with diastolic blood pressure, eGFR and NT-proBNP ($p = 0.05$, $p = 0.04$, $p < 0.001$; respectively). sRAGE was univariably associated with diabetes mellitus, heart rate, NYHA functional class, NT-proBNP, HbA1c, total cholesterol, E/A ratio, LVEF and peak VO_2 ($p = 0.01$, $p = 0.03$, $p = 0.04$, $p = 0.03$, $p = 0.004$, $p = 0.02$, $p < 0.001$, $p = 0.01$, $p = 0.07$; respectively). Multivariable linear regression showed that sRAGE was independently associated with diabetes mellitus and E/A ratio ($r^2 = 0.31$, $p < 0.001$).

Table 3 Correlations plasma AGEs, tissue AGEs and sRAGE

	Pentosidine	sRAGE	Skin AF	Age	HbA1c	MDRD
CML	r = 0.68 P < 0.001	r = 0.10 P = 0.33	r = 0.16 P = 0.12	r = 0.26 P = 0.01	r = 0.04 P = 0.74	r = -0.42 P < 0.001
Pentosidine		r = 0.08 P = 0.44	r = 0.18 P = 0.09	r = 0.14 P = 0.18	r = 0.19 P = 0.06	r = -0.20 P = 0.06
sRAGE			r = -0.00 P = 0.99	r = 0.07 P = 0.53	r = 0.29 P = 0.01	r = -0.14 P = 0.17
Skin AF				r = 0.43 P < 0.001	r = 0.24 P = 0.02	r = -0.20 P = 0.06
Age					r = 0.22 P = 0.04	r = -.41 P < 0.001
HbA1c						r = 0.01 P = 0.91

CML, N^{ε}-(carboxymethyl)lysine; sRAGE, soluble receptor of advanced glycation end-product; skin AF, skin autofluorescence; HbA1c, haemoglobin A1c

Discussion

The present study showed that levels of plasma AGEs, tissue AGE and sRAGE were mildly elevated in this cohort of systolic HF patients. In contrast to our expectations, the advanced glycation end-product crosslink breaker alagebrium did not change any AGE-related parameter.

AGEs have been proposed as an underlying mechanism causing diastolic HF through increased rigidity, by creating crosslinks, and/or by the binding capacity of AGEs to the receptor of AGE (RAGE).(1) When AGE is interacting with RAGE, NADPH-oxidase and transforming growth factor-β (TGF-β) are activated and upregulated, causing an increase in inflammation and fibrosis, which induces cardiovascular dysfunction.(1,21) By breaking crosslinks, structural changes that are related to diastolic dysfunction might be reversed.(9) The effect of the AGE-crosslink breaker alagebrium (ALT-711) has been studied both in animals and humans. In experimental studies, alagebrium reacts with and cleaves covalent, AGE-derived protein crosslinks.(9) By cleaving crosslinks alagebrium might be able to reverse structural changes that are related to diastolic dysfunction, resulting in a decrease in tissue AGEs.(9,12,13) It furthermore leads to decreased tissue stiffness.(14,16,22) After the AGE crosslinks have been removed, AGEs may potentially shift to plasma, resulting in an increase in plasma AGEs. This increase in plasma AGEs can cause upregulation of RAGE, and may therefore also increase sRAGE levels.(21) sRAGE is the C-truncated secretory isoform of RAGE that circulates in plasma.(5) sRAGE has been proposed to bind up AGEs and interferes with AGEs' ability to bind and activate RAGE, and thereby slow the progression of inflammation and fibrosis.(5-8)

However in the current study alagebrium did not change the levels of CML, pentosidine, tissue AGE (measured with skin AF) or sRAGE.

A possible explanation could be the selection of the patient population. In this study, only patients with LVEF ≤ 0.45 were included for two reasons. First, since this is a small study, the risk of including non-HF patients needed to be decreased. Therefore, patients should have a clear diagnosis of HF with clear signs of systolic dysfunction. Second, diastolic function is even more impaired in patients with a reduced LVEF, when compared to patients with a preserved LVEF. No attempt was made to select isolated diastolic HF patients, in whom the largest effect of alagebrium would be expected.

A second explanation for the result of the BENEFICIAL trial could be the low tissue AGE levels, possibly caused by the low percentage of diabetic patients and the selection of systolic HF patients. Therefore the contribution of AGE-crosslinking to diastolic dysfunction, in this population, might have been low.(2,23) This is further supported by the low amount

of sRAGE levels in this HF population compared to other HF trials.(5,24) The plasma AGE pentosidine was also low compared to a previously reported study in patients with diastolic dysfunction.(23) However, plasma CML levels were slightly elevated in the studied population compared to another study of systolic HF patients.(3)

The largest effect of AGE crosslink breakers can be expected in elderly patients with diabetes or renal failure, who will have a high amount of AGEs and may therefore benefit the most from AGE interventions. The AGE crosslink breaker TRC4186 is currently under investigation for use in diabetic HF patients (eudraCT number of this trial is 2008-006237-27)

Conclusion

In this randomized placebo controlled trial, the AGE-breaker alagebrium did not change levels of plasma AGEs, tissue AGE and sRAGE in patients with systolic HF. These results support the absence of positive effects on exercise capacity and cardiac function, which could be allocated to alagebrium.

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Tissue advanced glycation end-products are associated with diastolic function and aerobic exercise capacity in diabetic heart failure patients

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Abstract

Aims

Advanced glycation end products (AGEs) are increased in patients with diabetes and are associated with diastolic dysfunction through the formation of collagen crosslinks in the heart. The association among AGEs, diastolic function, and aerobic capacity in heart failure (HF) patients with and without diabetes is, however, unknown. We therefore studied the association among tissue AGEs, diastolic function, and aerobic capacity in patients with HF with or without diabetes.

Methods and results

In chronic HF patients (with and without left ventricular systolic dysfunction), tissue AGEs [skin autofluorescence (AF)], diastolic function (echocardiographic mean E' and E/E'), and aerobic capacity [peak oxygen uptake (VO_2) on cardiopulmonary exercise testing] were obtained. A total of 49 diabetics and 156 non-diabetics were included. Diabetics were older and had more cardiovascular risk factors, but left ventricular ejection fractions (LVEF) were similar. Tissue AGEs were higher in diabetics compared with non-diabetics (2.8 ± 0.8 vs. 2.3 ± 0.7 a.u.; $P < 0.001$). Furthermore, there was a correlation between tissue AGEs and mean E' ($r = -0.30$; $P < 0.001$, after adjustment for age, $r = -0.21$; $P = 0.004$). Aerobic capacity was significantly lower in diabetic patients with HF (peak VO_2: 17.4 ± 5.1 vs. 21.7 ± 6.1 mL/min/kg; $P = 0.001$), even after adjustment for age and LVEF. Peak VO_2 was related to skin AF ($P = 0.03$), independent of age, diabetes, LVEF, and New York Heart Association functional class.

Conclusion

Patients with diabetes and HF have similar LVEF but poorer exercise capacity compared with non-diabetic HF patients. Our data suggest that these findings might be explained by the observed association among tissue AGE levels, diastolic function, and exercise capacity.

Introduction

The prevalence of heart failure (HF) is increasing, partly due to an ageing population, but also due to an increasing prevalence of diabetes.(1,2) Patients with diabetes have an increased risk of developing HF, independent of their higher age.(3) This could be explained by a higher risk of myocardial infarction, vascular dysfunction, and diastolic dysfunction in diabetes.(4) However, several other metabolic factors have been postulated to explain the increased risk of HF in patients with diabetes.

Advanced glycation end products (AGEs) are formed during a non-enzymatic reaction between proteins and sugar residues, called the Maillard reaction.(5,6) Advanced glycation end products accumulation occurs during life and enhanced AGE accumulation plays a role in the pathophysiology of chronic HF, renal dysfunction, and diabetic complications.(5) Accumulation of AGEs affects the physiological properties of proteins and multiple vascular and tissue changes via the interaction of AGEs.(5)

Increased AGEs have been found in diabetic patients and in patients with HF.(7) Wu et al. and Loimaala et al.(8,9) showed that diabetics have a more impaired diastolic function and poorer exercise capacity compared with non-diabetics. Furthermore, Parthenakis et al.(10) showed that, in HF patients, diastolic function is the most important predictor of exercise intolerance measured with cardiopulmonary exercise testing. We hypothesized that elevated AGEs are related to diastolic function and exercise capacity, and therefore studied the association among tissue AGEs, diastolic function, and aerobic exercise capacity in HF patients with and without diabetes.

Methods

Patients and study design

The present study analysed the data of all patients who were screened at the outpatient clinic for a double-blind, placebo-controlled, randomized trial in patients with chronic HF (BENEFICIAL). The study design, baseline characteristics, and inclusion and exclusion criteria have been published elsewhere.(11) Briefly, patients with New York Heart Association (NYHA) II–IV stable HF for at least 3 months and left ventricular ejection fraction (LVEF) of ≤0.45 were eligible for the study. Main exclusion criteria were the inability of patients to undergo exercise testing, cardiac resynchronization therapy, pacemaker therapy, active, and or treated malignancies within 12 months prior to inclusion, and clinically significant renal disturbance. Patients receiving treatment directed towards glucose regulation were considered as diabetics. A total of 205 patients were screened at the outpatient department. In each patient, echocardiography and skin autofluorescence (AF; skin AF is a validated non-invasive method to study tissue AGEs) (12,13) were planned, and blood was drawn for laboratory analysis. These measurements were performed on the same day. Before randomization, a first rehearse cardiopulmonary aerobic capacity test was performed during a preceding visit to familiarize the patient with the procedures (n = 133). The BENEFICIAL study was approved by the local medical Ethics Committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. All subjects gave written informed consent.

Skin autofluorescence

Tissue AGE accumulation was assessed using a validated skin AF reader (AGE reader; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands) as described previously. (12,13) In short, the AGE reader illuminates a skin surface of ~ 2 cm^2, guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation of ~ 370 nm). Light from the skin is measured with a spectrometer in the 420-600 nm range, using 200 µm glass fibres. The value of skin AF is calculated as the ratio of the light intensity in the 420-600 nm wavelength range and the light intensity in the 300-420 nm wavelength range. Meerwaldt et al.(13) have shown that repeated skin AF measurements on one day show an overall Altman error rate of 5.03%. Intra-individual seasonal variance shows an Altman error rate of 5.87%. The differences between repeated measurements do not alter depending on the skin AF level. Skin AF was measured at the volar side of the lower arm at ~ 10-15 cm below the elbow fold. The measurement was performed three times at a healthy skin site (i.e. without visible vessels, scars, or other skin abnormalities) and an average was calculated.

Echocardiography

Patients underwent two-dimensional echocardiography.(14) Echocardiography was performed by experienced technicians using a VIVID 7 system (General Electric, Horten, Norway) with a 2.5-3.5 MHz probe. Measurements included left ventricular and atrial dimensions. Diastolic function was evaluated with peak early (E) and late (A) diastolic filling velocities, isovolumetric relaxation time (IVRT), left atrial end-diastolic volume (LAEDV), left atrial end-systolic volume (LAESV), and deceleration time (Dct) of the early peak filling. Early diastolic tissue velocity (E') was measured in colour-coded tissue Doppler imaging (CC-TDI) and calculated in pulse-wave tissue Doppler imaging with the formula described by Hummel et al.(15) Furthermore, left ventricular mass (LVmass) and left atrial volume index (LAVI) were calculated.(14) E' values were measured on the lateral and septal wall areas using CC-TDI. E/E' was calculated by dividing the E by the average E'. Systolic dysfunction was determined by Simpson's LVEF and defined as LVEF ≤ 0.45. When Simpson's LVEF could not reliably be determined, LVEF was estimated visually.(14) Diastolic function was categorized as no, mild, moderate, or severe diastolic dysfunction according to the Recommendations for the Evaluation of Left Ventricular Diastolic Function by the American Society of Echocardiography.(14)

Cardiopulmonary aerobic capacity testing

Cardiopulmonary aerobic capacity testing was performed according to the modified Bruce protocol, which increases the workload more gradually than the Bruce protocol. The first stage is performed at 1.7 m.p.h. and 0% grade, the second stage at 1.7 m.p.h. and 5% grade, and the third stage corresponds to the first stage of the Bruce protocol.(16) Each exercise test started with an acclimatization period of standing on the treadmill. A standard 12-lead electrocardiogram was recorded continuously during exercise testing. Blood pressure was registered on a regulatory basis using a manual cuff sphygmomanometer. Patients were encouraged to continue the exercise until their peak oxygen uptake (VO_2) was reached, they became uncomfortably symptomatic, or discontinuation was indicated for safety reasons. Oxygen uptake, carbon dioxide production, and minute ventilation were measured using breath-by-breath gas analysis. Peak VO_2 is determined as an average value of the two highest VO_2 values at peak performance, expressed as mL/min/kg and as mL/min/fat free mass as well as a percentage of predicted peak oxygen consumption.

Statistical analysis

Variables that were normally distributed were expressed as mean ± SD, whereas non-normally distributed variables were expressed as median (25-75% inter-quartile range). Nominal parameters were expressed as n (%). Differences in characteristics between patient groups were analysed using a t-test, when the parameter was normally distributed or a Mann-Whitney U-test when normality was not met. Correlation coefficients were calculated using Pearson's correlation coefficients. Based on earlier studies on predictors of exercise capacity in diabetic patients, we predefined variables that were entered in the multivariate model. Variables were considered in the multivariate models when a P-value <0.1 was obtained in univariate regression. Variables that did not retain significance in the multivariable regression analysis were subsequently removed from the model (backward selection). To test whether the model was appropriate and whether assumptions for linear regression were met, the model was tested for colinearity, interaction terms, and lack-of-fit analysis with variance. Residuals were tested for normality of distribution. Analyses were performed with SPSS 16.0.2 (SPSS Inc., Chicago, IL, USA). All statistical tests were two sided and a P-value <0.05 was considered statistically significant.

Results

Patient characteristics

Baseline characteristics of the study population are summarized in table 1. Mean age was 63 ± 12 years, 168 (82%) were male, and diabetic patients were older (P = 0.001). Skin AF was higher in patients with diabetes (2.8 ± 0.8 vs. 2.3 ± 0.7 a.u., P < 0.001). There was no difference in LVEF (0.35 ± 0.11 vs. 0.37 ± 0.12, P = 0.26) or LVmass (219 ± 65 vs. 234 ± 81 g, P = 0.26) between patients with and without diabetes. Diastolic function was classified as mild in 88 (44%) patients, moderate in 38 (19%) patients, and severe in 5 (3%) patients, which was not statistically significant between diabetic and non-diabetic patients. However, mean E' was lower (4.8 ± 1.9 vs. 5.6 ± 2.0 cm/s, P = 0.02) and LAEDV was larger (36 (27 - 59) vs. 49 (30 - 74) mm, P = 0.05) in patients with diabetes. Multivariable linear regression showed that skin AF was determined by age, diabetes, smoking, and estimated glomerular filtration rate (table 2).

Table 1 Baseline characteristics

Variables	Total population (n = 205)	No Diabetes (n = 156)	Diabetes (n = 49)	p -value
Age(years)	63 ± 12	61 ± 12	68 ± 10	0.001
Sex (male), n (%)	168 (82)	129 (83)	39 (80)	0 62
Race (caucasian), n (%)	202 (99)	155 (99)	47 (96)	0.08
Cardiovascular risk factors, history of				
Hypercholesterolaemia, n (%)	151 (74)	106 (68)	45 (92)	0.001
Hypertension, n (%)	76 (37)	51 (33)	25 (51)	0.02
Duration of hypertension, years	15 ± 12	14 ± 11	16 ± 13	0.52
Smoking, n (%)	169 (83)	131 (84)	38 (78)	0.31
Body mass index, kg/m²	28 ± 5	28 ± 4	30 ± 6	0.01
Rhythm, n (%)				0.26
Sinus rhythm	179 (87)	138 (88)	41 (84)	
Atrial flutter/fibrillation	22 (11)	14 (9)	8 (16)	
Other	4 (3)	4 (3)	0 (0)	
Systolic blood pressure, mmHg	121 ± 19	119 ± 19	126 ± 19	0.05
Diastolic blood pressure, mmHg	73 ± 10	74 ± 11	72 ± 8	0.12
Heart rate, b.p.m.	70 ± 14	67 ± 12	79 ± 16	<0.001
Etiology of HF, n (%)				0.46
Ischaemic	131 (64)	95 (61)	36 (74)	
Idiopathic	47 (23)	39 (25)	8 (16)	
Rhythm disturbances	12 (6)	10 (6)	2 (4)	
Other	15 (7)	12 (8)	3 (6)	
NYHA functional class, n (%)				0.03
II	121 (59)	97 (62)	24 (49)	
III	73 (36)	54 (35)	19 (39)	
IV	11 (5)	5 (3)	6 (12)	
Skin AF, a.u.	2.4 ± 0.8	2.3 ± 0.7	2.8 ± 0.8	<0.001
Laboratory assessments				
Hb, mmol/l	8.8 ± 0.9	8.9 ± 0.8	8.5 ± 0.9	0.03
eGFR, mL/min/1.73 m²	75 ± 23	77 ± 21	69 ± 26	0.05
HbA1c, %	5.8 (5.6 - 6.6)	5.7 (5.5 - 6.0)	7.1 (6.6 - 7.9)	<0.001
Echocardiography				
LVEDD, ms	57 ± 9	57 ± 9	56 ± 9	0.76
LVESD, ms	45 ± 11	45 ± 11	44 ± 11	0.95
E/A ratio	0.87 (0.67 - 1.16)	0.88 (0.72 - 1.17)	0.74 (0.61 - 1.08)	0.11
IVRT,ms	100 ± 22	98 ± 22	105 ± 24	0.09
Dct, ms	219 ± 63	222 ± 62	207 ± 66	0.16

Table 1 Continued

Variables	Total population (n = 205)	No Diabetes (n = 156)	Diabetes (n = 49)	p -value
Mean E', cm/sec	5.4 ± 2.0	5.6 ± 2.0	4.8 ± 1.9	0.02
E/E'	13 (10 - 18)	12 (9 - 17)	14 (11 - 20)	0.02
LAEDV, mm	38 (27 - 64)	36 (27 - 59)	49 (30 - 74)	0.05
LAESV, mm	69 (51 - 94)	68 (50 - 90)	76 (53 - 98)	0.23
LAVI. ml/m²	34 (26 - 46)	32 (25 - 45)	37 (27 - 49)	0.26
LVmass, gram	223 ± 69	219 ± 65	234 ± 81	0.26
LVEF	0.37 ± 0.12	0.37 ± 0.12	0.35 ± 0.11	0.26
Peak VO_2, mL/min/kg (n = 133)	20.8 ± 6.1	21.7 ± 6.1	17.4 ± 5.1	0.001
Medication use, n (%)				
ACE-inhibitor/ARB	190 (93)	144 (92)	46 (94)	0.71
Beta-blocker	182 (89)	138 (89)	44 (90)	0.80
Diuretics	116 (57)	80 (51)	36 (74)	0.01
Aldosteron antagonists	56 (27)	39 (25)	17 (35)	0.18

HF, Heart Failure; NYHA, New York Heart Association; skin AF, skin autofluorescence; Hb, hemoglobin; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin type A1c; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; LAVI, left atrial volume index; IVRT, isovolumetric relaxation time; Dct, deceleration time; LAEDV, left atrial end-diastolic volume; LAESV, left atrial end systolic volume; LVmass, left ventricular mass; LVEF, left ventricular ejection fraction; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker

Table 2 Determinants of skin autofluorescence (skin AF)

Variable	Univariate			Multivariate (r = 0.51 r^2= 0.26 p < 0.001)		
	ß	B (95%CI)	p-value	ß	B (95%CI)	p-value
Age	0.38	0.03 (0.02;0.03)	<0.001	0.26	0.02 (0.01;0.03)	<0.001
Diabetes	0.31	0.55 (0.31;0.79)	<0.001	0.21	0.38 (0.16;0.61)	0.001
BMI	-0.07	-0.01 (-0.03;0.01)	0.31			
Smoking	0.18	0.23 (0.05;0.40)	0.01	0.20	0.25 (0.10;0.41)	0.002
eGFR	0.32	0.71 (0.41;1.02)	<0.001	-0.18	-0.01 (-0.01;-0.00)	0.01

Skin AF, skin autofluorescence; BMI, body mass index; eGFR, estimated glomerular filtration rate

Association between tissue advanced glycation end products and diastolic function

Table 3 shows baseline characteristics of patients with low or high tissue AGEs. Skin AF was measured in 197 (96%) of 205 patients. Patients with skin AF above the mean were older ($P < 0.001$), more often had diabetes ($P < 0.001$), a more impaired diastolic function measured with mean E' ($P < 0.001$), and aerobic exercise capacity ($P < 0.001$). Figure 1 demonstrates the relation between higher tissue AGEs and mean E'. Skin AF was significantly correlated with diastolic function measured with mean E' ($r = -0.310$; $P < 0.001$, after adjustment for age, $r = -0.212$; $P = 0.004$). Multivariable linear regression analysis showed that skin AF remained independently associated with diastolic function ($P < 0.001$, Table 4), after adjustment for potential confounders, such as age, LVEF, hypercholesterolaemia, hypertension, duration of hypertension, and body mass index.

Tabel 3 Baseline characteristics divided by mean skin autofluorescence

Variables	Total population (n = 197)	Skin AF < mean (n = 110)	Skin AF > mean (n = 87)	p-value
Age, years	63±12	59 ± 11	68 ± 11	<0.001
History of diabetes, n (%)	46 (23)	14 (13)	32 (37)	<0.001
Smoking, n (%)	165 (84)	90 (82)	75 (86)	0.43
Systolic blood pressure, mmHg	121 ± 19	120 ± 19	122 ± 20	0.58
Diastolic blood pressure, mmHg	73 ± 10	75 ± 11	71 ± 10	0.01
Aetiology, ischaemic, n (%) of HF	126 (64)	63 (57)	63 (72)	0.03
NYHA functional class, n (%)				0.003
II	114 (58)	74 (67)	40 (46)	
III/IV	83 (42)	36 (33)	47 (54)	
Laboratory assessments				
Hb, mmol/l	8.8 ± 0.9	9.0 ± 0.7	8.4 ± 0.9	<0.001
eGFR, mL/min/1.73 m^2	74 ± 22	79 ± 19	68 ± 24	0.001
HbA1c, %	5.8 (5.6 - 6.6)	5.7 (5.5 - 6.2)	6.0 (5.6 - 6.9)	0.003
Echocardiography				
E/E'	12 (10 - 18)	11 (9 - 14)	14 (10 - 21)	<0.001
Mean E', cm/sec	5.5 ± 2.0	6.0 ± 1.8	4.8 ± 1.9	<0.001
LAEDV, mm	39 (28 - 65)	37 (27 - 62)	41 (28 - 68)	0.65
LAVI, mL/m^2	34 (25 - 46)	32 (25 - 44)	36 (27 - 48)	0.19
LVEF, (mean ± SD)	0.37 ± 0.11	0.37 ± 0.11	0.36 ± 0.12	0.40
Peak VO_2, mL/min/kg (n = 131)	20.5 ± 6.1	22.4 ± 6.0	17.6 ± 5.1	< 0.001

NYHA, new york heart association; Hb, hemoglobin; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin type A1c; LVEF, left ventricular ejection fraction

Figure 1 Relation between mean E' and skin autofluorescence (Skin AF)

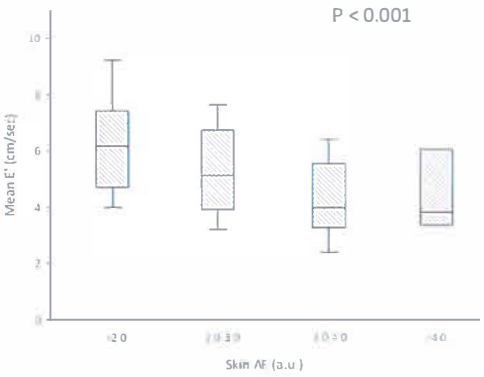

Table 4 Determinants of mean early diastolic tissue velocity (mean E')

Variable	Univariate			Multivariate (r = 0.46 r^2=0.22, p<0.001)		
	ß	B (95%CI)	p	ß	B (95%CI)	P
Skin AF	-0.31	-0.79 (-1.15;-0.43)	<0.001	-0.26	0.70 (-1.06; -0.34)	<0.001
Age	-0.32	-0.05 (-0.07;-0.03)	<0.001			
History of ischaemia	0.18	0.74 (0.15;1.33)	0.01			
LVEF	0.38	0.06 (0.04;0.09)	<0.001	0.36	0.06 (0.04-0.09)	<0.001
Hypercholesterolaemia	-0.12	-0.55 (-1.21;-0.11)	0.10			
Body mass index	0.11	0.04 (-0.02;0.10)	0.14			
Hypertension	-0.12	-0.48 (-1.08;0.11)	0.11			
Duration of hypertension, per 5 years	-0.24	-0.21 (-0.41-0.04)	0.05			
History of diabetes	0.20	-0.95 (-1.65;-0.25)	0.01			

Skin AF, skin auto fluorescence; LVEF, left ventricular ejection fraction

Table 5 determinants of peak oxygen uptake

Variable	Univariate			Multivariate (r=0.58 r^2= 0.34 p< 0.001)		
	ß	B (95%CI)	p	ß	B (95%CI)	p
Skin AF	-0.38	-3.04 (-4.34;-1.74)	<0.001	-0.18	-1.47 (-2.79;-0.14)	0.03
Age	-0.32	-0.17 (-0.26;-0.08)	<0.001	-0.20	-0.11 (-0.19;-0.03)	0.01
Diabetes	-0.29	-4.3 (-6.8;-1.9)	0.001	-0.18	-2.70 (-5.00;-0.42)	0.02
LVEF	0.34	0.22 (0.12;0.33)	<0.001	0.29	0.19 (0.09;0.28)	<0.001
NYHA	-0.29	-3.62 (-5.72;-1.52)	0.001	-0.17	-2.18 (-4.07;-0.29)	0.02
mean E'	0.17	0.54 (0.00;1.07)	0.05			

Peak VO_2, peak oxygen uptake; Skin AF, skin autofluorescence; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association

Association between tissue advanced glycation end products and exercise capacity

Exercise capacity was measured in 133 (65%) of 205 patients and was lower in diabetics compared with non-diabetics (17.6 ± 5.1 vs. 22.4 ± 6.0 mL/min/kg, P < 0.001). Table 5 shows that peak VO_2 was related to skin AF (P = 0.03), independent of age, diabetes, LVEF, and NYHA functional class.

Discussion

The present study shows that tissue AGEs are higher in diabetic HF patients compared with patients with HF without diabetes and are independently associated with diastolic function and cardiopulmonary aerobic exercise testing. This is the first study that describes the association among tissue AGEs, diastolic function, and aerobic exercise capacity in patients with HF with and without diabetes.

Patients with diabetes have an increased risk of developing HF, and multiple factors might explain this relation.(17,18) Recently, it was suggested that AGEs might be an important contributor to the development of HF among patients with diabetes.(5) The association between AGEs and HF can be explained by two main mechanisms. First, AGEs affect the physiological properties of proteins in tissues by creating crosslinks. Second, AGEs cause multiple vascular and tissue changes via the interaction with AGE receptors. Exposure to AGEs can cause a significant delay in calcium re-uptake causing diastolic dysfunction. Others have shown that exercise capacity in diabetic patients is more impaired compared with non-diabetics.(8,9) In the present study, we extended these findings to patients with HF. Several studies have shown the importance of diastolic dysfunction as an independent predictor of reduced exercise capacity, even after adjusting for age.(8-10,19) In the present study, we found similar systolic function and LVmass, but lower early diastolic velocities, suggestive of poorer diastolic function, although other diastolic parameters of diastolic dysfunction did not show significant differences between patients with and without diabetes. Interestingly, we found diastolic function to be a predictor of exercise capacity. After adjustment for tissue AGEs, the predictive value of diastolic function became non-significant. The most important novel finding of the present study is, however, that we provided evidence for an association between tissue AGEs and both mean E' and exercise capacity, suggesting that AGEs might explain the impaired diastolic function (measured with mean E'), and therefore, the poorer exercise capacity in diabetic HF patients.

Two limitations should be noted. First, tissue AGEs were measured non-invasively at the volar side of the right arm. Although this measurement has been validated against AGEs that are present in the skin, the skin AF reader has not been validated directly against AGEs present in the heart. Second, we studied diastolic function in patients with systolic HF. However, diastolic function is frequently present in patients with systolic HF, and Bursi et al.(20) showed that diastolic function was even more impaired in patients with systolic HF compared with patients with diastolic HF. If AGEs provide a pathophysiological explanation for impaired diastolic function and poorer exercise capacity in diabetic HF patients, this might provide a novel treatment option. Although diastolic dysfunction is common and strongly related to symptoms, there is no standardized treatment to improve diastolic function. (21-25) The stiffness due to accumulation of AGEs could also be a target for intervention with AGE breakers by reversing structural changes that are related to diastolic function. The largest effect of AGE breakers can be expected in older patients with diabetes or renal failure, who will have a high amount of AGEs and may, therefore, benefit the most from AGE intervention. Several AGE cross-link breakers, such as alagebrium and TRC4186, are currently under investigation for use in diabetic and non-diabetic HF patients.

Conclusion

Patients with diabetes and HF have similar LVEF but poorer exercise capacity compared with non-diabetic HF patients. Our data suggest that these findings might be explained by the observed association among tissue AGE levels, diastolic function, and exercise capacity.

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Advanced glycation end-products and outcome in heart failure patients with preserved and reduced ejection fraction

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Abstract

Introduction

Advanced glycation end products (AGEs) are increased in patients with heart failure (HF). We studied the predictive value of plasma AGEs N^{ε}-(carboxymethyl)lysine (CML) and pentosidine in a large HF population.

Methods

In 580 patients hospitalized with HF plasma AGEs were measured before discharge when patients were clinically stable. Patients were followed for a period of 18 months. Primary endpoint of interest was a composite of death and HF admission. CML was determined by liquid chromatography mass spectrometry and pentosidine by high performance liquid chromatography.

Results

Mean age was 71 ± 11 years, 62 % was male, and mean left ventricular ejection fraction (LVEF) was 0.32 ± 0.14. At baseline, mean CML level was 2.16 ± 0.73 µmol/L and median pentosidine was 0.043 (0.030 - 0.074) µmol/L. CML and pentosidine levels, were independently related to composite end-point (hazard ratio (HR) = 1.20 per SD, 95% CI:1.05 - 1.37; p=0.01 and HR = 1.15 per SD, 95% CI:1.00 - 1.31; p = 0.045; respectively) and HF hospitalization (HR = 1.27 per SD, 95% CI:1.10 - 1.48; p = 0.001 and HR = 1.27 per SD, 95%CI:1.10 - 1.47; p = 0.001, respectively). Furthermore, CML levels were independently related to increased mortality (p = 0.006).

Discussion

In HF patients, both CML and pentosidine predict HF hospitalization and the combined primary end-point (mortality or HF hospitalization). Furthermore CML was significantly and independently associated with a higher risk for mortality.

Introduction

Increased prevalence of heart failure (HF) is related to aging of the population and an improved survival after myocardial infarction.(1) The number of HF hospitalizations has also increased.(2) Advanced glycation end-products (AGEs) increase during aging, and are suggested to be a pathophysiological mechanism for the increased prevalence of HF in the elderly population.(3) AGEs are end-products formed by oxidative and non-oxidative reactions between sugars and proteins.(4) AGEs form cross-links with long-living tissue proteins, which cause them to accumulate in the body over time. Through decreased compliance of the heart and the vasculature, AGE accumulation is considered to be related to the onset and progression of HF.(3) In a smaller group of systolic HF patients, we recently showed that plasma AGE N^{ε}-(carboxymethyl)lysine (CML) was related to prognosis.(5) However, after adjustment for renal function, this relation subsided. In another study, serum pentosidine was related to severity of HF, although this was not adjusted for important potential confounders, such as haemoglobin and gender.(6) In the present sub-study, we studied the independent predictive value of the plasma AGEs CML and pentosidine, in a large group of HF patients.

Methods

The COACH (the Coordinating study evaluating Outcomes of Advising and Counseling in Heart failure) study was a multicenter, randomized, controlled study to compare basic and intensive support in patients hospitalized for HF. The primary outcome was a composite end-point of HF hospitalization and all-cause mortality. The design and primary results of the study have previously been published.(7,8) Briefly, patients hospitalized for HF with evidence for structural underlying disease were eligible for the study. Main exclusion criteria were invasive interventions within the last 6 months or planned during the following 3 months and ongoing evaluation for heart transplantation. A total of 1023 patients were included in the study in 17 participating centers, and follow-up was performed at 1, 6, 12 and 18 months after discharge. From an unselected subgroup of 580 patients, blood samples were taken before discharge. Baseline characteristics of the 1023 subjects in the main trial did not differ significantly from the 580 patients included in this sub-study. HF patients were divided into preserved and reduced Left ventricular ejection fraction (LVEF). An LVEF above 0.40 was classified as preserved LVEF. Blood samples were collected at baseline and at 6 months after discharge. In 16 centers additional blood samples were collected. The study was approved by the Medical Ethical Committee and all subjects gave written informed consent.

Determination of N^{ε}-(carboxymethyl)lysine

N^{ε}-(carboxymethyl)lysine (CML) was determined by stable-isotope-dilution liquid chromatography tandem mass spectrometry (LC-MS/MS).(9) In short, CML was liberated from plasma proteins by acid hydrolysis after addition of deuterated CML as internal standards. Chromatographic separation was performed by gradient-elution reversed-phase chromatography with a mobile phase containing 5 mmol/L nonafluoropentanoic acid as ion-pairing agent. Mass transitions of 205.1 → 84.1 CML, and 209.1 → 88.1 and 223.1 → 88.1 for the respective internal standards was monitored in positive-ion mode. CML was separated by baseline resolution with a total analysis time of 21 min. Within-day and between-day coefficients of variation were < 4.4% and < 3.2%.

Determination of pentosidine levels

Pentosidine levels were measured by high performance liquid chromatography (HPLC).10) Plasma proteins were hydrolysed in 6 N HCL. Detection of pentosidine is done based on its own fluorescence characteristics using fluorimetric detection (E_x = 325 nm, E_m = 385 nm). Separation is done, with a run-to-run time of 30 min, on a C_{18} Allspehere ODS-II column with a citric acid acetonitrile buffer. This detection enables sensitive and specific determination of protein bound pentosidine in plasma with detection limit of 2.2 nmol/l or 0.02 pmol/mg.

Statistical analysis

Variables which were normally distributed were expressed as mean ± SD, whereas non-normally distributed variables are expressed as median (25% to 75% interquartile range) Nominal parameters were expressed as n (%). Differences in characteristics between patient groups were analyzed using a t-test when the parameter is normally distributed or a Mann-Whitney U test when normality is not met. Correlation coefficients were calculated using Pearson's correlation coefficients. Linear regression analysis was used to assess the determinants of CML and pentosidine. Variables were considered in the multivariable models when P-value < 0.10 was obtained in univariate regression. Variables that did not retain significance were subsequently removed from the model (backward selection). To test whether the model was appropriate and whether assumptions for linear regression were met, the model was tested for collinearity, interaction terms, and lack-of fit analysis with variance. Residuals were tested for normality of distribution. Kaplan-Meier survival plots were constructed by dividing CML and pentosidine by median at baseline to study the influence of plasma AGEs on all cause mortality. Cox proportional hazard regression was used to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) and to determine univariable and multivariable predictors for the primary end-point, hospitalization for HF and all cause mortality. Univariable factors with $p < 0.10$ were identified and entered in the multivariable model to assess the impact of plasma AGEs. Variables which not retained significance in the multivariable analysis were subsequently removed from the model (backward selection).There were no violations found for the assumption of proportional hazards. Furthermore, interaction analysis was performed to investigate the differential prognostic effect of AGEs in reduced vs. preserved LVEF and diabetics vs. non-diabetics patients. CML and log pentosidine were centered by subtracting their average value. Analyses were performed with SPSS 16.0.2 (SPSS Inc., Chicago, IL, USA). All statistical tests were two sided and p-value < 0.05 was considered statistically significant.

Table 1 Baseline characteristics before discharge

Variable	Total population (n = 580)	Survivors (n = 415)	Non-Survivors (n = 165)	p -value
Age, years	71 ± 11	70 ± 11	74 ± 10	<0.001
Sex (male), n (%)	361 (62)	251 (61)	110 (67)	0.17
LVEF	0.32 ± 0.14	0.33 ± 0.14	0.32 ± 0.14	0.49
NYHA, n (%)				0.001
II	270 (47)	213 (52)	57 (35)	
III/IV	307 (53)	200 (48)	107 (65)	
Systolic Blood pressure (mmHg)	118 ± 21	119 ± 22	115 ± 20	0.03
Diastolic blood pressure (mmHg)	69 ± 12	70 ± 12	67 ± 13	0.008
Laboratory assessments				
Hb (mmol/L)	8.2 ± 1.2	8.3 ± 1.2	7.9 ± 1.2	0.003
eGFR (ml/min/1.73m^2)	54 ± 20	57 ± 20	47 ± 19	< 0.001
CML (µmol/L)	2.16 ± 0.73	2.05 ± 0.60	2.42 ± 0.94	<0.001
Pentosidine (µmol/L)	0.04 (0.03 - 0.07)	0.04 (0.03 - 0.06)	0.05 (0.03 - 0.10)	< 0.001
NT-proBNP (pmol/L)	2530 (1309 - 5721)	2186 (1127 - 4383)	4547 (2135 - 9950)	< 0.001
History of, n (%):				
Hypertension	250 (43)	178 (43)	72 (44)	0.87
Smoking	98 (17)	71 (17)	27 (16)	0.78
Diabetes	177 (31)	109 (26)	68 (41)	<0.001
Af/Afl	266 (46)	175 (42)	91 (55)	0.005
Medication at discharge, n (%)				
Beta-blocker	389 (67)	288 (69)	101 (61)	0.06
Ace-i/ARB	477 (82)	353 (85)	124 (75)	0.005
Diuretics	555 (96)	394 (95)	161 (98)	0.16

LVEF = left ventricular ejection fraction; NYHA = new york heart association; Hb = haemoglobin; eGFR = estimated glomerular filtration rate; CML = Nε-(carboxymethyl)lysine; NT-proBNP = N-terminal-pro brain natriuretic peptide; Af/Afl = atrial fibrillation/ atrial flutter; ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker

Table 2 Patient characteristics divided by left ventricular ejection fraction

Variable	LVEF ≤ 0.40 (n = 335)	LVEF > 0.40 (n = 136)	p-value
Age, years	69 ± 11	74 ± 10	<0.001
Sex (male), n (%)	222 (66)	75 (55)	0.02
LVEF	0.25 ± 0.07	0.51 ± 0.09	<0.001
CML (µmol/L)	2.16 ± 0.69	2.07 ± 0.66	0.24
Pentosidine (µmol/L)	0.05 (0.03 - 0.08)	0.04 (0.03 - 0.07)	0.20

LVEF = left ventricular ejection fraction; CML = Nε-(carboxymethyl)lysine

Results

Patients characteristics

Baseline characteristics of the study population are presented in table 1. Mean age was 71 ± 11 years, 62% was male and mean LVEF was 0.32 ± 0.14. Diabetes mellitus was present in 177 (31%) patients. Out of the 580 patients, 165 (28%) died. Non-survivors were older ($p < 0.001$), had a higher New York Heart Association (NYHA) functional class ($p = 0.001$), lower haemoglobin levels ($p = 0.003$), more renal dysfunction ($p < 0.001$), higher NT-proBNP ($p < 0.001$) and higher CML ($p < 0.001$) and pentosidine levels ($p < 0.001$). Furthermore, non-survivors were more often diabetic ($p < 0.001$) and had more frequently atrial fibrillation or atrial flutter ($p = 0.005$). The patient characteristics are presented by dividing the population in preserved vs. reduced LVEF (table 2). No significant differences were found in baseline CML and pentosidine levels between the two patient groups ($p = 0.24$ and $p = 0.20$, respectively). In both preserved and reduced LVEF, non-survivors had higher pentosidine levels compared to survivors (0.052 (0.034 - 0.118) µmol/L vs 0.035 (0.026 - 0.058) µmol/L; $p = 0.003$ and 0.054 (0.033 - 0.099) µmol/L vs. 0.043 (0.029 - 0.067) µmol/L; $p = 0.005$, respectively). In HF patients with reduced LVEF non-survivors had a higher CML level compared to survivors (2.41 ± 0.88 µmol/L vs. 2.05 ± 0.57 µmol/L; $p < 0.001$, respectively). There was no significant difference in CML levels in relation to mortality in patients with preserved LVEF (2.25 ± 0.85 µmol/L in non-survivors vs. 2.01 ± 0.57 µmol/L in survivors; $p = 0.14$).

Plasma AGEs

Mean levels of CML at baseline were 2.16 ± 0.73 µmol/L and median levels of pentosidine at baseline were 0.043 (0.030 - 0.074) µmol/L. Patients with higher baseline plasma AGEs CML and pentosidine were on average older ($p < 0.001$ and $p = 0.05$, respectively), had higher NYHA functional classes ($p = 0.02$ and $p < 0.001$, respectively), higher NT-proBNP levels (both $p < 0.001$), decreased eGFR levels (both $p < 0.001$) (table 3). Next to that, plasma AGEs CML and pentosidine in the total population were significantly higher in non-survivors compared to survivors (both $p < 0.001$). Furthermore, correlations existed between CML and eGFR levels ($r = -0.33$; $p < 0.001$) and pentosidine and eGFR levels ($r = -0.23$; $p < 0.001$). Univariate regression analysis showed an association between pentosidine and diabetes ($p = 0.03$), which did not retained significant in multivariate regression analysis (table 4). No association was found between CML and diabetes ($p = 0.18$; table 5). An interaction was found between eGFR and log NT-proBNP (after correction $r = 0.42$; $p < 0.001$) and between death and eGFR (after correction $r = 0.42$; $p < 0.001$). Kaplan Meier survival plots for combined primary end-point, hospitalization for HF and all cause mortality showed that CML and pentosidine levels above median at baseline were associated with worse prognosis (figure 1-3).

Table 3 Baseline characteristics stratified for CML and pentosidine by median

Variable	CML < median (n=291)	CML > median (n=289)	p- value	Pentosidine < median (n = 290)	Pentosidine > median (n = 287)	p- value
Age, years	69 ± 12	73 ± 10	<0.001	70 ± 11	72 ± 11	0.05
Sex (male), n (%)	171 (59)	190 (66)	0.08	184 (63)	177 (62)	0.66
Death, n (%)	65 (22)	100 (35)	0.001	64 (22)	101 (35)	<0.001
NYHA, n (%)			0.02			<0.001
II	150 (52)	120 (42)		159 (55)	111 (39)	
III/IV	140 (48)	167 (58)		129 (45)	175 (61)	
Laboratory assessments						
Hb (mmol/L)	8.3 ± 1.3	8.0 ± 1.2	0.008	8.3 ± 1.2	8.0 ± 1.3	0.01
eGFR (ml/min/1.73m^2)	60.2 ± 20.0	47.9 ± 18.3	<0.001	59.7 ± 19.1	48.3 ± 19.4	<0.001
NT-proBNP (pmol/L)	2099 (1183 - 4245)	3370 (1455 - 8020)	<0.001	1903 (1050 - 3804)	4033 (1892 - 8712)	<0.001
History of, n (%)						
Smoking	61 (21)	37 (13)	0.009	51 (18)	47 (16)	0.70
Diabetes	93 (32)	84 (29)	0.45	82 (28)	95 (33)	0.21

NYHA, new york heart association; Hb, haemoglobin; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro-brain natriuretic peptide

Table 4 linear regression models, with CML as dependent variables

	Univariate			Multivariate r= 0.40 r^2= 0.16 p < 0.001		
Variables	ß	B (95%CI)	p	ß	B (95%CI)	p
Age	0.14	0.01 (0.00 ; 0.02)	0.001			
Death	0.23	0.37 (0.24 ; 0.50)	<0.001	0.11	0.17 (0.04-0.31)	0.01
NYHA	0.15	0.23 (0.11 ; 0.34)	<0.001			
Hb	-0.15	-0.10 (-0.17; -0.03)	0.01			
eGFR	-0.33	-0.01 (-0.02; -0.01)	<0.001	-0.24	-0.01 (-0.01;-0.01)	<0.001
Log NT-proBNP	0.29	0.40 (0.29-0.52)	<0.001	0.19	0.27 (0.15-0.39)	<0.001
Diabetes	0.06	0.09 (-0.04;0.22)	0.18			
LVEF	-0.05	-0.00 (-0.00;0.00)	0.32			
Smoking	-0.07	-0.14 (-0.30-0.02)	0.09	-0.09	-0.17 (-0.31;-0.02)	0.03

NYHA, new york heart association; Hb, haemoglobin; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro-brain natriuretic peptide; LVEF, left ventricular ejection fraction

Table 5 linear regression model, with pentosidine as the dependent variable

	Univariate			Multivariate r = 0.44 r^2= 0.19 p < 0.001		
Variables	ß	B (95%CI)	p	ß	B (95%CI)	p
Age	0.09	0.0 (0.0 ; 0.0)	0.03			
Death	0.22	0.14 (0..09 ; 0.20)	<0.001			
NYHA	0.22	0.13 (0.08 ; 0.18)	<0.001	0.14	0.08 (0.04 ; 0.13)	0.001
Hb	-0.18	-0.04 (-0.07 ; -0.02)	0.002			
eGFR	-0.30	-0.00 (-0.01 ; -0.00)	<0.001	-0.19	-0.00 (-0.00 ; -0.00)	<0.001
Log NT-proBNP	0.37	0.21 (0.17 ; 0.26)	<0.001	0.30	0.17 (0.12 ; 0.22)	<0.001
Diabetes	0.09	0.06 (0.01 ; 0.11)	0.03			
LVEF	-0.06	-0.00 (-0.00 ; 0.00)	0.20			
Smoking	-0.03	-0.03 (-0.09 ; 0.04)	0.42			

NYHA, new york heart association; Hb, haemoglobin; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro-brain natriuretic peptide; LVEF, left ventricular ejection fraction

Figure 1 Kaplan Meier survival curves for combined primary end-point of all-cause mortality and hospitalization for heart failure for pentosidine (A) and N^{ε}-(carboxymethyl)lysine (CML) (B)

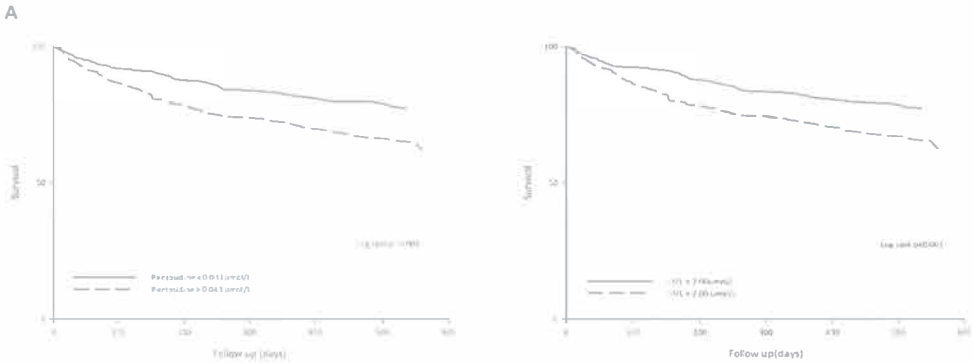


Figure 2 Kaplan Meier survival curves for hospitalization for heart failure for pentosidine (A) and N^{ε}-(carboxymethyl)lysine (CML) (B)

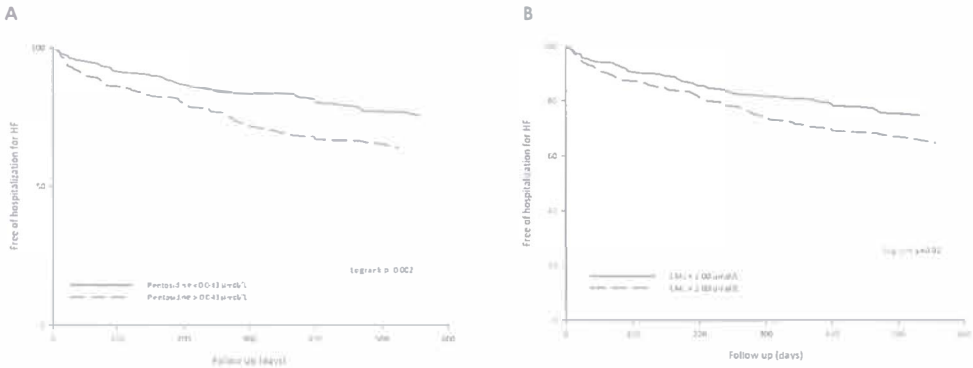
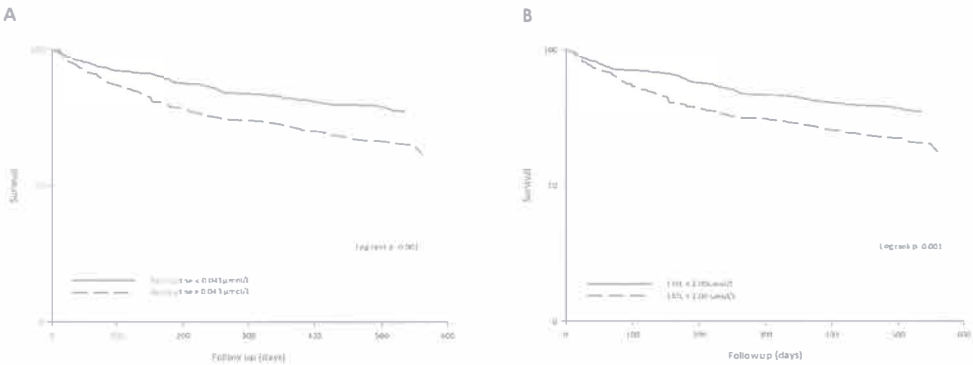


Figure 3 Kaplan Meier survival curves for all cause mortality for pentosidine (A) and N^{ε}-(carboxymethyl)lysine (CML) (B)



Prognosis

In univariable Cox regression analysis, CML and log pentosidine were associated with the combined primary end-point (both $p < 0.001$, table 6a and 6b), HF hospitalization ($p < 0.001$ and $p = 0.02$ respectively; table 7a and 7b) and mortality (both $p < 0.001$; table 8a and 8b). Multivariable Cox regression analysis showed that higher CML and log pentosidine levels were related to the primary end-point (CML: hazard ratio (HR) = 1.20 per SD, 95% CI: 1.05 - 1.37; $p = 0.001$ and pentosidine: HR = 1.15 per SD, 95% CI: 1.00 - 1.31; $p = 0.045$). This result was independent of known predictors (age, sex, eGFR, NT-proBNP, haemoglobin, LVEF and diabetes) (table 6a and 6b). In univariable Cox regression analysis, higher CML and log pentosidine levels were associated with HF hospitalization (CML: HR = 1.27 per SD, 95% CI: 1.10 - 1.48); $p = 0.001$ and pentosidine: HR = 1.27 per SD, 95% CI: 1.20 - 1.47; $p = 0.001$)(table 7a and 7b). Finally, high CML levels at baseline were related to mortality (HR = 1.24 per SD, 95% CI: 1.07 - 1.45; $p = 0.006$, table 8a). Interaction analysis did not show a differential prognostic effect of AGEs in diabetic and non-diabetic patients or in patients with a reduced or preserved LVEF.

Interaction analysis

There were no significant interactions regarding the primary endpoint, HF hospitalization and all cause mortality for: CML and LVEF ($p = 0.89$, $p = 0.40$ and $p = 0.30$ respectively), pentosidine and LVEF ($p = 0.56$, $p = 0.59$ and $p = 0.51$, respectively), CML and diabetic history ($p = 0.86$, $p = 0.47$ and $p = 0.60$, respectively) and pentosidine and diabetic history ($p = 0.90$, $p = 0.44$ and $p = 0.39$, respectively).

Table 6a Cox regression for combined primary end-point of all-cause mortality and hospitalization for heart failure with CML included as variable

Variable	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, per SD	1.37 (1.19-1.58)	<0.001	1.20 (1.02-1.41)	0.03
Smoking	0.91 (0.70-1.20)	0.91		
Sex	0.84 (0.69-1.03)	0.10		
eGFR, per SD	0.63 (0.55-0.37)	<0.001	0.80 (0.68-0.94)	0.01
Log NT-proBNP, per SD	1.59 (1.38-1.82)	<0.001	1.36 (1.17-1.58)	<0.001
Hb, g/dL	0.82 (0.74-0.92)	<0.001		
CML, per SD	1.41 (1.26-1.57)	<0.001	1.20 (1.05-1.37)	0.01
LVEF, per SD	1.02 (0.89-1.18)	0.78		
Diabetes mellitus	1.67 (1.36-2.03)	<0.001	1.67 (1.28-2.18)	<0.001

eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro-brain natriuretic peptide; Hb, haemoglobin; CML, N$^{\varepsilon}$-(carboxymethyl)lysine; LVEF, left ventricular ejection fraction

Table 6b Cox regression for combined primary end-point of all-cause mortality and hospitalization for heart failure with pentosidine included as variable

Variable	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, per SD	1.37 (1.19-1.58)	<0.001	1.21 (1.03-1.42)	0.02
Smoking	0.91 (0.70-1.20)	0.91		
Sex	0.84 (0.69-1.03)	0.10		
eGFR, per SD	0.63 (0.55-0.37)	<0.001	0.79 (0.67-0.93)	0.004
Log NT-proBNP, per SD	1.26 (1.17-1.36)	<0.001	1.35 (1.16-1.57)	<0.001
Hb, g/dL	0.82 (0.74-0.92)	<0.001		
Log pentosidine, per SD	1.40 (1.25-1.57)	<0.001	1.15 (1.00-1.31)	0.045
LVEF, per SD	1.02 (0.89-1.18)	0.78		
Diabetes mellitus	1.67 (1.36-2.03)	<0.001	1.65 (1.26-2.15)	<0.001

eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro-brain natriuretic peptide; Hb, haemoglobin; LVEF, left ventricular ejection fraction

Table 7a Cox regression for hospitalization for heart failure with CML included as variable

Variable	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, per SD	1.26 (1.05-1.50)	0.01		
Smoking	0.71 (0.46-1.11)	0.71		
Sex	1.39 (1.04-1.86)	0.03		
eGFR, per SD	0.63 (0.53-0.76)	<0.001	0.71 (0.59-0.86)	<0.001
Log NT-proBNP, per SD	1.39 (1.17-1.65)	<0.001		
Hb, g/dL	0.82 (0.69-0.96)	0.01		
CML, per SD	1.37 (1.17-1.61)	<0.001	1.27 (1.10-1.48)	0.001
LVEF, per SD	1.11 (0.93-1.32)	0.24		
Diabetes mellitus	1.94 (1.44-2.61)	<0.001	1.93 (1.40-2.67)	<0.001

eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro-brain natriuretic peptide Hb, haemoglobin; CML, N-(carboxymethyl)lysine; LVEF, left ventricular ejection fraction

Table 7b Cox regression for hospitalisation for heart failure with pentosidine included as variable

Variable	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, per SD	1.26 (1.05-1.50)	0.01		
Smoking	0.71 (0.46-1.11)	0.71		
Sex	1.39 (1.04-1.86)	0.03		
eGFR, per SD	0.63 (0.53-0.76)	<0.001	0.71 (0.59-0.86)	<0.001
Log NT-proBNP, per SD	1.39 (1.17-1.65)	<0.001		
Hb, g/dL	0.82 (0.69-0.96)	0.01		
Log pentosidine, per SD	1.16 (1.03-1.32)	0.02	1.27 (1.10-1.47)	0.001
LVEF, per SD	1.11 (0.93-1.32)	0.24		
Diabetes mellitus	1.94 (1.44-2.61)	<0.001	1.89 (1.36-2.62)	<0.001

eGFR, estimated glomerular filtration rate NT-proBNP, N-terminal-pro-brain natriuretic peptide; Hb, haemoglobin; LVEF, left ventricular ejection fraction

Table 8a Cox regression for all cause mortality with CML included as variable

Variable	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, per SD	1.56 (1.36-1.81)	<0.001	1.35(1.11-1.64)	0.003
Smoking	1.05 (0.69-1.58)	0.83		
Sex	1.26 (0.91-1.74)	0.16		
eGFR, per SD	0.60 (0.52-0.69)	<0.001		
LogNT-proBNP, per SD	1.90 (1.60-2.26)	<0.001	1.74(1.45-2.09)	<0.001
Hb, g/dL	0.78 (0.66-0.92)	0.003		
CML, per SD	1.49 (1.32-1.69)	<0.001	1.24 (1.07-1.45)	0.006
LVEF, per SD	0.97 (0.85-1.10)	0.64		
Diabetes mellitus	1.75 (1.28-2.39)	<0.001	1.65 (1.20-2.27)	0.002

eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro-brain natriuretic peptide; Hb, haemoglobin; CML, Nε-(carboxymethyl)lysine; LVEF, left ventricular ejection fraction

Table 8b Cox regression for all cause mortality with pentosidine included as variable

Variable	Univariable		Multivariable	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age, per SD	1.56 (1.36-1.81)	<0.001	1.38 (1.13-1.69)	0.001
Smoking	1.05 (0.69-1.58)	0.83		
Sex	1.26 (0.91-1.74)	0.16		
eGFR, per SD	0.60 (0.52-0.69)	<0.001	0.81 (0.67-0.98)	0.03
LogNT-proBNP, per SD	1.90 (1.60-2.26)	<0.001	1.73 (1.45-2.08)	<0.001
Hb, g/dL	0.78 (0.66-0.92)	0.003		
Log Pentosidine, per SD	1.23 (1.13-1.35)	<0.001		
LVEF, per SD	0.97 (0.85-1.10)	0.64		
Diabetes	1.75 (1.28-2.39)	<0.001	1.61 (1.16-2.22)	0.004

eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal-pro-brain natriuretic peptide; Hb, haemoglobin; LVEF, left ventricular ejection fraction

Discussion

The present study showed that higher plasma CML and pentosidine levels were significantly and independently associated with HF hospitalization and combined end-point (death and HF hospitalization). Furthermore CML levels at baseline were associated with a higher mortality rate. This is the largest study so far that describes the independent prognostic value of CML and pentosidine levels.

Plasma AGEs accumulate with age. AGEs affect the physiological properties of tissues by creating cross-links, which causes ventricular and arterial stiffness.(3,5,11-13) Multiple vascular and tissue changes also occur via the interaction with AGE receptors. Next to this does exposure to AGEs also cause significant delays in calcium reuptake causing diastolic dysfunction. This process can be accelerated by several diseases, such as diabetes mellitus, chronic kidney disease, HF and atherosclerosis.(3,5,13-16) Due to these vascular and tissue changes, it seems reasonable to believe that AGEs are related to the onset and progression of heart failure. The findings of the present study further support this concept.

Only three other studies reported on the prognostic value of plasma AGEs in heart failure. In a smaller group of systolic HF patients (n = 102), we previously showed that plasma AGE CML was related to prognosis of HF, but after adjustment for renal function the relation subsided. (5) In the present study we confirmed this observation of a correlation between plasma AGEs and eGFR. The current study, however, had the power to study the predictive value of CML and pentosidine independent of kidney function, haemoglobin and gender, which are known predictors of mortality in HF patients.(17,18) In another smaller HF population (n = 141), Koyama et al. found that serum pentosidine was related to severity of HF and it was an independent risk factor to predict adverse clinical outcomes in HF patients.(6) Adverse clinical outcomes were defined as a combined end-point of cardiac death or rehospitalization due to progressive HF. In the present study, we demonstrated that CML and pentosidine were independent predictors for HF hospitalization and the combined primary end-point. In addition, CML, but not pentosidine, was also related to mortality. In another study of 160 HF patients, pentosidine was an independent predictor for cardiac events, defined as a combined end-point of cardiac death and rehospitalization, in patients with HF ($p = 0.012$).(19) In this study CML was not investigated. To our knowledge, this is the first study that investigated the predictive capabilities of both CML and pentosidine on HF hospitalization, mortality and its combined primary end-point (HF hospitalization and mortality).

AGE-accumulation is associated with reduced survival in patients with HF, diabetes and/or renal failure and may therefore be a target for intervention. One method to target these adverse effects of AGE-accumulation is via AGE crosslink breakers. In the BENEFICIAL trial (a double-blind, placebo-controlled, randomized trial, evaluating the efficacy and safety of alagebrium (ALT-711) in patients with systolic chronic HF), the effect of the AGE-breaker alagebrium, on exercise capacity and cardiac function in systolic HF patients, was studied.(20) The results showed no significant changes on either exercise capacity or cardiac function. An explanation for these unexpected results could be found in the study population. The study population consisted of well treated HF patients with relatively low NT-proBNP levels, probably related to the selection of patients that were able to undergo exercise testing. On baseline, patients had fairly good exercise capacities, which make it difficult to demonstrate significant improvements in exercise capacity. Moreover, severely symptomatic, elderly HF patients with serious co-morbidities, like diabetes or renal failure, limiting their exercise capacity, were not included in this study. These severely symptomatic patients will expectedly have a high amount of AGEs may therefore benefit the most from AGE intervention. The AGE crosslink breaker TRC 4186 is currently under investigation for use in diabetic HF patients (eudraCT number of this trial is 2008-006237-27).

Limitations

A limitation of this study should be addressed. In this study, the predictive value of plasma AGEs was studied, independently of known predictors of mortality, in HF patients. However, diastolic function was not taken into account. Previous research has shown an association between AGEs and diastolic function.(11,21)

Conclusion

The present study showed that in HF patients, both CML and pentosidine predict HF hospitalization and the combined primary end-point (mortality or HF hospitalization). Furthermore CML was significantly and independently associated with a higher risk for mortality. Together with its pathophysiological properties, these findings support the concept that AGEs are related to the onset and progression of HF

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Discussion

Introduction

This thesis aimed to study the pathophysiological role of advanced glycation end-products (AGEs) in the development and progression of heart failure (HF), and the effect of the AGE-crosslink breaker alagebrium, on exercise tolerance, plasma and tissue AGE levels and soluble receptor of AGE (sRAGE) levels in chronic systolic HF patients.

The prevalence of patients with chronic HF is increasing mainly due to the ageing population and the increasing prevalence of diabetes.(1,2) Chronic HF may coincide with either depressed (systolic) or preserved (diastolic) left ventricular ejection fraction (LVEF).(3) In almost all HF patients (either with depressed or preserved LVEF), diastolic dysfunction is present.(4-7) Patients with diastolic dysfunction can have well known symptoms of HF. Systolic HF patients almost always have both diastolic and systolic dysfunctions, while diastolic HF patients only have isolated diastolic dysfunction.(7) Diastolic HF exists when the ventricle is unable to accept an adequate blood volume during diastole (given normal diastolic volumes and pressures to maintain an appropriate stroke volume).(7) This is caused by a decrease in ventricle relaxation and/or an increase in ventricular stiffness.(7) Even though diastolic dysfunction is common and strongly related to HF symptoms, the current understanding of diastolic HF from a pathophysiological point of view is low. Several mechanisms underlying diastolic HF have been proposed.(8-12) One of the potential mechanisms is the deleterious effect of AGEs. AGEs induce structural modifications in the extracellular matrix, causing ventricular stiffness, which occurs in patients with diastolic dysfunction.(8)

Advanced glycation end-products

AGEs are a group of compounds, formed by a set of oxidative and non-oxidative reactions between proteins and sugar residues, called a Maillard reaction.(8,13,14) This Maillard reaction is a slow reaction and initiates when protein amino groups are exposed to sugar adducts, and proceeds via reversible Schiff base adducts to more stable Amadori products (e.g. HbA1c). This proceeds with the formation of stable and irreversible AGE compounds, for example N^{ε}-(carboxymethyl)lysine (CML) and pentosidine, via the molecular re-arrangement of the Amadori products.(8,13) The final step, resulting in the formation of AGEs, is catalyzed by oxidative stress.(8) AGEs themselves cause acceleration of oxidation, and thereby catalyzing this final step, creating a vicious circle. The degradation products of AGEs and its precursors are excreted via the kidney.(15) Patients with renal dysfunction have decreased clearance of AGEs and its precursors, and thereby accumulation of AGEs can occur.

Another aspect of AGE is its binding capacity to the receptor of AGE (RAGE).(8) RAGE is a cell surface molecule that is expressed in a variety of cell lines. When AGE is interacting with RAGE, NADPH-oxidase and transforming growth factor-β (TGF-β) are activated and upregulated, causing an increase in inflammation and fibrosis. AGE-accumulation can cause upregulation of RAGE, resulting in an increase in AGE-binding capacity to RAGE. This creates a potential vicious circle.(16) However, RAGE has a secretory isoform, sRAGE, that circulates in plasma.(17) sRAGE has been proposed to bind up AGEs and interferes with AGEs' ability to bind and activate RAGE, and thereby slow the progression of inflammation and fibrosis. (17-20)

Advanced glycation end-products increasing mechanisms

In HF patients, AGEs may be increased via five pathways.

First, AGEs accumulate in general during ageing. This accumulation occurs throughout the body, including the skin, neural, vascular and renal tissue.(21,22)

Second, direct AGE intake, from smoking cigarettes and eating heated, cooked or roasted food products, can be responsible for AGE-accumulation.(8,23,24)

Third, AGE accumulation is caused by reduced clearance capability of AGE degradation products. After modification or degradation of AGEs, in proximal tubuli, the resulting products are eventually cleared in urine.(25-27) Patients with renal dysfunction have a reduced renal clearance and consequently reduced clearance of AGE degradation products. This can result in AGE accumulation.

Fourth, an increase in AGE formation is noticed in the presence of oxidative stress, which is especially the case in patients with renal dysfunction.(28) This effect is enhanced by the AGE induced acceleration of the oxidation process, creating a "chicken and egg" situation. (29)

The fifth pathway suggests that AGE accumulation depends on glucose concentration (elevated in i.e. diabetes) in combination with protein turnover rate.(30) Glucose exposed protein amino groups react via Maillard reactions, producing AGE's. The rate of formation of one of the intermediate products of this reaction, the so called Amadori products, is directly proportional to the glucose concentration and is rate determining for the full reaction.(30)

Effects of advanced glycation end-products on cardiac and renal function

Accumulated AGEs can have multiple negative effects on the body. For this thesis three are of importance; renal dysfunction, myocardial diastolic dysfunction (potentially resulting in HF) and the "cardiorenal syndrome".

AGEs induce renal dysfunction via the following two mechanisms;
First, AGE stimulate endothelial cells in the kidney to release inflammatory mediators (vascular cell adhesion molecule-I and intercellular adhesion molecule-I).(25,31,32). This can cause potential tissue damage in the kidney resulting in renal dysfunction
Second, AGE-accumulation causes excessive cross-linking, resulting in increasing vascular and tissue rigidity, thereby causing renal dysfunction.

AGEs induce myocardial diastolic dysfunction via three proposed pathways. In the first, AGE-accumulation causes excessive cross-linking, increasing vascular and tissue rigidity, thereby causing diastolic dysfunction.(8,11,33,34) In the second, the product of a reaction between AGE and RAGE, as previously mentioned, causes an increase in inflammation and fibrosis, potentially resulting in diastolic dysfunction.(16) In the third, RAGE is exposed to AGEs, causing a significant delay in calcium reuptake. As a consequence, the duration of the repolarisation phase of the cardiac contraction increases, subsequently causing diastolic dysfunction.(8,14)

Diastolic dysfunction predisposes to HF development. In patients with chronic HF the co-existence of renal dysfunction is common (often referred to as "cardiorenal syndrome"). In this co-existence, chronic dysfunction in one organ (in this case either heart or kidney) may induce chronic dysfunction in the other organ (either kidney or heart), potentially creating a "chicken and egg" situation. This results in this case in the deterioration of both renal and myocardial functions.(35) One can speculate that the AGE related dysfunction in both kidney and heart are enhanced in patients with the cardiorenal syndrome.

Treatment

AGE-accumulation has been associated with a reduced survival in patients with diabetes, renal failure and HF, and may therefore be a target of intervention. The adverse effects of AGE-accumulation can be targeted in several ways.

First, AGE formation can be reduced by angiotensin II type 1 receptor blockers (ARBs).(36-38) ARBs prevent the production of reactive carbonyl and dicarbonyl compounds (RCOs), which are critical precursors of AGEs.(36-39) However, our group showed that the ARB eprosartan did not decrease levels of AGEs, within 6 months, in patients with hypertension and diastolic dysfunction.(40)

Second, AGE intake can also increase AGE-accumulation. A reduction of smoking and low-AGE diets reduces AGE levels in blood.(8,23,24)

Third, AGE crosslink breakers might be able to reverse AGE-induced crosslinks in the extracellular matrix. AGEs have been proposed as an underlying mechanism causing diastolic HF through increased rigidity, by creating crosslinks, and by causing a delay in calcium reuptake.(8) The effect of the AGE-crosslink breaker alagebrium (ALT-711) has been studied both in animals and humans. In experimental studies, alagebrium reacts with and cleaves covalent, AGE-derived protein crosslinks.(41) By cleaving crosslinks, alagebrium might be able to reverse structural changes that are related to diastolic dysfunction, resulting in decreased tissue AGEs(41-43) and tissue stiffness.(44-46) After AGE crosslinks have been removed, AGEs may shift to plasma, potentially increasing plasma AGEs. Accumulation of plasma AGEs can cause upregulation of RAGE. If AGE interacts with RAGE, an increase in inflammation and fibrosis occurs, inducing cardiovascular dysfunction.(8)

Finally, by blocking the interaction between AGE and RAGE, the negative cascade inducing cardiovascular dysfunction can be prevented. AGE-receptor interactions can be prevented in several ways, however, most of the inhibitors have multiple sites of action.(47) By adding sRAGE, AGEs and AGE precursors can be scavenged from the circulation.(47,48) sRAGE is an isoform of RAGE, that circulates in plasma.(17) sRAGE has been proposed to have an atherosclerotic-protective function, in particular by acting as a decoy for AGEs.(17-19) Furthermore, inhibition of an AGE-RAGE interaction may be accomplished by blocking RAGE with antibodies, preventing the AGE-mediated damage.(47,48)

BENEFICIAL

In chapter 2 and 3, the design and the results of the BENEFICIAL trial are described. The BENEFICIAL trial (a double-blind, placebo-controlled, randomized trial, evaluating the efficacy and safety of alagebrium (ALT-711) in patients with systolic chronic HF) was conducted to investigate whether the AGE-breaker alagebrium improves exercise capacity and cardiac function in patients with HF.(49) In this thesis we showed that the AGE-breaker alagebrium did not improve exercise capacity or cardiac function in systolic HF patients.

There are several possible explanations for this result. The selection of the study population could have influenced our findings. The study population consisted of well treated HF patients with relatively low NT-proBNP levels at baseline, probably related to the selection of patients that were able to undergo exercise testing. On baseline, patients had fairly good exercise capacities, which make it difficult to demonstrate significant improvements in exercise capacity. Moreover, severely symptomatic, high aged patients with serious co-morbidities limiting their exercise capacity, were not included in this study. Results can therefore not readily be applied to more severely symptomatic HF patients.
Furthermore, only patients with systolic HF (LVEF ≤ 0.45) were included in this study for two reasons. First, to maintain sufficient power in this small study, the risk of including non-HF patients needed to be decreased. Therefore, patients should have a clear diagnosis of HF with clear signs of systolic dysfunction. Second, as previously mentioned in the introduction, diastolic function is more impaired in patients with a reduced LVEF, compared to patients with a preserved LVEF. No attempt was made to select isolated diastolic HF patients, in whom the largest effect of alagebrium could be expected.

A second explanation for the result of the BENEFICIAL trial could be the low tissue AGE levels, possibly caused by the low percentage of diabetic patients, patients with fairly good renal function, and the selection of systolic HF patients. Therefore the contribution of AGE-crosslinking to diastolic dysfunction, in this population, might have been low. This is supported by the low amount of sRAGE and plasma AGE pentosidine in this HF population, compared to other HF trials.(17,50,51)

The lack of effects of AGE-breaker alagebrium in the BENEFICIAL study is further supported by findings described in chapter 4. In 96 out of 102 patients of the BENEFICIAL study, plasma AGEs CML and pentosidine, sRAGE and tissue AGE levels were measured. These measurements were conducted before start of treatment (alagebrium or placebo) and after 36 weeks of treatment. Alagebrium did not reduce tissue AGEs, nor did it increase

plasma and sRAGE levels. These results, therefore, supports the absence of positive effects on exercise capacity and cardiac function, in systolic HF patients treated with alagebrium.

Prognostic value of AGEs

Several factors have been established as independent predictors for survival in HF patients, among which are LVEF, New York Heart Association (NYHA) functional class, anemia and renal function.(11,52-54) AGEs increase during ageing, and are also suggested to be a pathophysiological mechanism for the increased prevalence of HF in the elderly population. (8) AGE accumulation is considered to be related to the onset and progression of HF, through decreasing compliance of the heart and vasculature.(8)

Only three other studies reported on the prognostic value of plasma AGEs in HF. Our group showed that plasma CML was related to the severity and prognosis of HF patients (n = 102).(11) This relation subsided after correction for renal function, but a correlation between plasma AGEs and eGFR was found. In another HF population (n = 141), a relation was found between serum pentosidine and severity of HF. Furthermore serum pentosidine was an independent risk factor to predict adverse clinical outcomes in HF patients.(55) Adverse clinical outcomes were defined as a combined end-point of cardiac death or HF hospitalization. In the third study of HF patients (n = 160), pentosidine proved to be an independent predictor for cardiac events, defined as a combined end-point of cardiac death and hospitalization (p = 0.012).(50) In this study CML was not investigated. In chapter 6 we showed that in a large population of HF patients (n = 580), both CML and pentosidine predict HF hospitalization and the combined primary end-point (mortality or HF hospitalization). Furthermore CML was significantly and independently associated with a higher risk for mortality. These conclusions confirmed that the results shown in the three smaller HF populations were also applicable for a large HF population. Together with its pathophysiological properties, the findings in this chapter support the concept that AGEs are related to the onset and progression of HF.

Future perspectives

This thesis reports on some of the adverse clinical effects of AGE-accumulation and the safety and efficacy of the AGE-crosslink breaker alagebrium in chronic HF patients.

Future AGE research could target three main fields; adverse effects of AGEs, the prognostic value of AGEs and interactions between tissue and plasma AGEs for which outlines are given below.

Targeting adverse effects of AGEs

The adverse effects of AGE-accumulation can be targeted by aiming on different steps in the Maillard reaction. As discussed before, the Maillard reaction consists of oxidative and non-oxidative reaction steps between proteins and sugar residues, resulting in the formation of AGEs.(8,13,14) To reduce this formation different AGE intervention strategies have been identified.(8,47,48)

The three most promising steps for interfering with the negative effects caused by AGEs are presented below;
The first targets the AGE levels available in the body by treating HF patients with an AGE-crosslink breaker (e.g. alagebrium), which should reduce the excessive crosslinks formed by AGEs. Elderly HF patients in general but especially patients with either diabetes or renal failure, have high AGE levels and may therefore benefit the most from AGE intervention. Currently, the AGE crosslink breaker TRC 4186 is under investigation for use in diabetic HF patients (eudraCT number of this trial is 2008-006237-27).

The second and third methods are attempting to intervene in the AGE-RAGE interaction. The products, resulting from this AGE-RAGE interaction, induce cardiovascular dysfunction. By preventing this interaction, the AGE-mediated damage may be prevented. This can be achieved by either adding sRAGE, which scavenges AGEs and AGE precursors from the circulation, or by blocking RAGE with antibodies.(47,48) Although for both methods encouraging experimental results have been reported, clinical trials have yet to be reported. (56-60)

The prognostic value of AGEs

AGEs can cause ventricular and arterial stiffness as described before and therefore have a strong relation with diastolic dysfunction and HF. AGEs might be a pathophysiological factor for increased HF prevalence in patients. High plasma AGE levels (CML and pentosidine) were significantly and independently associated with a high mortality rate, compared with low AGE spiegels, in hospitalized HF patients, but no separate information is available on this association for preserved and reduced HF. However, from a pathophysiological point of view, differences are expected between reduced and preserved HF patients. In systolic HF patients eccentric hypertrophy exists, which, can be caused by ischemia (no AGE involvement). In diastolic HF patients concentric hypertrophy exists, which can be caused by an increase in ventricular and arterial stiffness, which, for example, can be caused by AGEs. It would therefore be of interest to study this interaction.

Interactions between tissues and plasma advanced glycation end-products levels

Only CML accumulation levels have been accurately measured in tissue biopsies of the skin, and roughly in human myocardial tissue, using immunostaining.(21) In addition, these AGE accumulation levels in both skin and serum have not been compared with those in myocardial tissue yet. However, a relation between increased skin and serum AGE levels with decreased diastolic function has been demonstrated.(11,14,51,61) However it has never been established whether the decreased heart function results from increased AGE accumulation in myocardial tissue. We therefore have initiated a cross-sectional pilot study to establish the relation between myocardial, skin and serum AGE levels. Patients are recruited from the cardio surgery waiting list. The primary end-point of this study is the relation between skin and myocardial AGE levels.

In conclusion, further research is needed to investigate AGEs . Especially the following areas are of interest; methods to mitigate the adverse effects of AGEs, the potential prognostic capabilities of AGEs and the interaction between AGEs in tissues with those in plasma. So there remain plenty of opportunities in an AGE-ing world!

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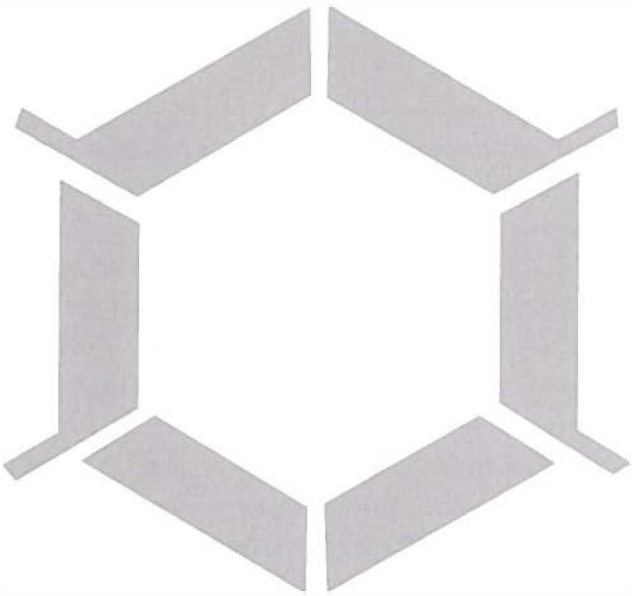


Nederlandse discussie

Inleiding

Dit proefschrift heeft de pathofysiologische rol van Advanced Glycation End-products (AGEs; versuikerde eiwitten) onderzocht bij het ontstaan en de progressie van hartfalen (HF). Tevens is bij patiënten met systolisch HF het effect van de AGE-breker alagebrium op het inspanningsvermogen, op de plasma en weefsel AGE spiegels én op de sRAGE spiegel (de oplosbare receptor van AGE) onderzocht.

De prevalentie van patienten met chronisch HF stijgt, voornamelijk door een steeds ouder wordende bevolking en door toename van de prevalentie van diabetes. Chronisch HF heeft twee verschijningsvormen, óf een verminderde (systolisch) óf een behouden (diastolisch) linker ventrikel ejectie fractie (LVEF). Bij bijna alle HF patiënten (zowel systolisch als diastolisch) is er sprake van diastolische disfunctie. Patienten met diastolische disfunctie hebben vaak de bekende HF symptomen. Patiënten met systolisch HF hebben bijna altijd zowel diastolische als systolische disfuncties, terwijl bij patiënten met diastolische HF alleen maar diastolische disfunctie voorkomt. Er wordt van diastolische HF gesproken wanneer de ventrikel niet meer in staat is om een adequate hoeveelheid bloed te accepteren tijdens de diastole (bij normale diastolische volumes en drukken, zodat een geschikt slagvolume wordt gehandhaafd). Dit wordt veroorzaakt door een afname in de ontspanning en/of een toename van de ventrikelstijfheid. Het huidige begrip van de pathofysiologische aspecten van diastolisch hartfalen is laag ondanks het veelvuldig voorkomen van diastolische disfunctie en de sterke relatie met HF symptomen. Er zijn verschillende mechanismen beschreven die diastolisch HF veroorzaken. Eén van deze mechanismes berust op de schadelijke effecten van AGEs. AGEs zorgen im=mers voor structurele wijzigingen in de extracellulaire matrix, waardoor de vertrikel verstijfd, zoals het geval is bij patiënten met diastolische disfunctie.

Advanced glycation end-products

AGEs zijn een groep verbindingen die gevormd kunnen worden via de zogenaamde Maillard reactie, een reeks van oxidatieve en niet-oxidatieve reacties tussen eiwitten en suiker residuen. Deze Maillard-reactie verloopt langzaam en wordt geinitieert door het blootstellen van aminogroepen van eiwitten aan suiker adducten. De reactie vervolgt via reversibele Schiff base adducten tot de stabielere Amadori producten (bijv. HbA1c). Via een moleculaire herschikking van deze Amadori producten worden de stabiele AGE verbindingen gevormd. Voorbeelden hiervan zijn N^{ε}-(carboxymethyl)lysine (CML) en pentosidine. Deze laatste stap, die resulteert in de daadwerkelijke vorming van AGEs, wordt gekatalyseerd door oxidatieve stress. AGEs verhogen de oxidatieve stress, waardoor de laatste stap van de reactie wordt gekatalyseerd, en een vicieuze cirkel ontstaat (figuur 1). De afbraakproducten en de

voorlopers van AGEs worden via de nieren uitgescheiden. Bij patiënten met een afgenomen nierfunctie kan door een verminderde klaring van ophoping van AGEs optreden.

AGEs zijn hiernaast in staat om een verbinding aan te gaan met de receptor van AGE (RAGE). RAGE is een molecuul dat zich op het celoppervlak bevindt en bij verschillende type cellen voorkomt. Bij de interactie tussen AGE en RAGE, worden NADPH-oxidase en de transforming growth factor-β (TGF-β) geactiveerd en gereguleerd wat resulteert in een toename van de ontstekingsreactie en fibrose. AGE-accumulatie kan leiden tot een toename van RAGE, en daarmee toename van de AGE-RAGE interactie. Hierdoor kan een vicieuze cirkel ontstaan. Er bestaat in het plasma ook een isoform van RAGE, sRAGE. Er wordt gedacht dat sRAGE, net als RAGE, een verbinding kan vormen met AGE. Hierdoor kan sRAGE in de interactie tussen AGE en RAGE interfereren, waardoor het de progressie van de ontstekingsreactie en fibrose vertraagd.

Advanced glycation end-products verhogende mechanismen

De AGE spiegel van HF patiënten kan worden verhoogd via vijf mechanismen (figuur 1).

Ten eerste stijgt de AGE spiegel in het lichaam tijdens het leven van de patient. Dit fenomeen treedt in het hele lichaam op, bijvoorbeeld in de huid, neurale-, vasculaire- en nierweefsel.

Ten tweede kan een stijging in de AGE spiegel veroorzaakt worden door de directe inname van AGEs door het roken van sigaretten en het eten van verwarmde, gekookte of gebraden voedsel.

Ten derde is een verminderde klaring van AGE afbraakproducten een directe oorzaak van AGE-accumulatie. De restproducten van AGEs worden, nadat AGEs in de proximale tubuli gemodificeerd of afgebroken zijn, via de urine uitgescheiden. Patiënten met nierdisfunctie hebben een verminderde klaring en kunnen daardoor minder AGE afbraakproducten uitscheiden, met AGE-accumulatie als gevolg.

Ten vierde stijgt de AGEs spiegel onder invloed van oxidative stress, wat vooral bij patiënten met een verminderde nierfunctie het geval is. Doordat AGEs het oxidatieproces versnellen wordt dit effect versterkt en ontstaat een vicieuze cirkel.

Ten vijfde hangt AGE accumulatie van de glucose concentratie af (verhoogd bij bijvoorbeeld diabetes) en de snelheid waarbij de eiwitten omgezet worden. Eiwitten, waarvan de aminogroep wordt blootgesteld aan glucose, vormen via de Maillard reactie AGEs. De snelheid waarmee AGEs gevormd worden is recht evenredig met de glucose concentratie en wordt bepaald door de vormingssnelheid van de Amadori producten, een van de tussenproducten in de Maillard reactie.

De effecten van AGEs op hart- en nierfunctie

Geaccumuleerde AGEs kunnen op meerdere manieren negatieve effecten hebben op het menselijk lichaam. Drie zijn er van belang in dit proefschrift; nierdisfunctie, myocardiale diastolische disfunctie (mogelijk resulterend in HF) en het "cardiorenale syndroom".

AGEs veroorzaken via twee mechanismen nierfalen;
In het eerste mechanisme stimuleren AGEs de endotheelcellen in de nieren zodat ontstekingsmediatoren (vascular cell adhesion molecule-l en intercellulaire adhesie molecuul-l) vrij worden gemaakt. Dit kan leiden tot schade aan de nier, met een vermindere nierfunctie tot gevolg.
In het tweede mechanisme veroorzaakt AGE-accumulatie overmatige cross-linking, wat resulteert in toenemende vasculaire en weefsel stijfheid, met een vermindere nierfunctie als gevolg.

AGEs veroorzaken via drie mechanismen myocardiale diastolische disfunctie. In het eerste mechanisme zorgt AGE-accumulatie voor overmatige cross-linking waardoor de stijfheid van bloedvaten en weefsels toeneemt, met diastolische disfunctie tot gevolg. In het tweede mechanisme zorgt de eerder vermelde reactie tussen AGEs en RAGE voor een toename in inflammatie en fibrose wat kan leiden tot diastolische disfunctie. In het derde mechanisme verminderd de heropname van calcium doordat RAGE blootgesteld wordt aan AGE. Als gevolg hiervan neemt de duur van de repolarisatie fase van de cardiale contractie toe, waardoor er sprake is van diastolische disfunctie.

Diastolische disfunctie geeft aanleiding tot de ontwikkeling van HF. Bij patiënten met chronisch HF komt vaak ook nierfalen voor (het zogenaamde "cardiorenaal syndroom"). Chronische disfunctie in één orgaan (in dit geval óf het hart óf de nieren) kan chronische disfunctie veroorzaken in het andere orgaan (in dit geval óf de nier óf het hart). Een vicieuze cirkel waarbij het falen van één orgaan het falen van een ander orgaan bespoedigd. Dit resulteert in dit geval in een verslechtering van zowel de nier- als de myocardiale functie. Het is mogelijk dat de AGE-gerelateerde disfunctie van zowel nieren als het hart aanwezig is in patienten met het cardiorenaal syndroom.

Behandeling

AGE-accumulatie is geassocieerd met een verminderde overlevingskans voor patiënten met diabetes, nierfalen of HF. Om deze reden is het een doelwit voor interventie. De schadelijke effecten van AGE-accumulatie kunnen op verschillende manieren worden aangepakt.

Ten eerste kan de vorming van AGEs worden verminderd door angiotensine II type 1 receptor blokkers (ARBs). ARBs verhinderen de vorming van reactieve carbonyl en dicarbonylverbindingen, voorlopers van AGE's. Onderzoek heeft echter aangetoond dat de ARB eprosartan niet binnen 6 maanden resulteert in een daling van de AGE spiegel in patiënten met hypertensie en diastolische disfunctie.

Ten tweede resulteert de reductie van directe AGE iname in een afname van de AGE spiegel. De AGE spiegel in het bloed vermindert bijvoorbeeld door te stoppen met roken en een AGE-arm dieet te volgen.

Ten derde zijn AGE crosslink-brekers mogelijk in staat om de door AGE-geïnduceerde verbindingen in de extracellulaire matrix te verbreken. Een mogelijk mechanisme dat diastolisch hartfalen veroorzaakt stelt dat AGEs crosslinks induceren, waardoor én de stijfheid in de weefsels toeneemt, én de heropname van calcium vertraagt. Het effect van de AGE-breker alagebrium (ALT-711) is onderzocht in zowel dieren als mensen. In experimentele studies reageertalagebrium met de door AGE veroorzaakte covalente crosslinks tussen eiwitten en splitst deze verbindingen. Door het verbreken van de crosslinks is alagebrium misschien in staat de aan diastolische disfunctie ten grondslag liggende structurele veranderingen terug te draaien, met een afname van weefsel AGEs en weefselstijfheid als gevolg. Nadat de AGE crosslinks zijn verbroken, zouden de AGEs zich naar het plasma kunnen verplaatsen, met een verhoging van plasma AGEs als gevolg. De toename van plasma AGEs kan leiden tot opregulatie van RAGE. De interactie tussen AGE en RAGE zorgt voor een toename van de ontstekingsreactie en fibrose, met mogelijk cardiovasculaire disfunctie tot gevolg.

Ten slote kan de vicieuze cirkel die cardiovaculaire disfunctie veroorzaakt worden gestopt door de interactie tussen AGE en RAGE te blokkeren. AGE-receptor interacties kunnen op verschillende manieren worden voorkomen en de werking van de meeste blokkers berusten op meerdere mechanismen tegelijk. AGE en AGE voorlopers kunnen worden weggevangen door toevoeging van sRAGE. sRAGE is een isovorm van RAGE en circuleert in het plasma. Er is gesuggereerd dat sRAGE een atherosclerotisch beschermende functie heeft, door als afleidingsdoel voor AGEs op te treden. Verder kan de AGE-RAGE interactie mogelijkwordentegengegaan door de werking van RAGE met antilichamen te blokkeren zodat de door AGEs veroorzaakte schade wordt voorkomen.

BENEFICIAL

In hoofdstuk 2 en 3 zijn het ontwerp en de resultaten van de BENEFICIAL studie beschreven. De BENEFICIAL studie (een dubbel-blind, placebo-gecontroleerde, gerandomiseerde studie naar de effectiviteit en veiligheid van alagebrium (ALT-711) bij patiënten met chronisch systolisch HF) onderzocht of de AGE-breker alagebrium, de inspanningscapaciteit en de cardiale functie verbeterde bij HF patiënten. In dit proefschrift wordt aangetoond dat alagebrium bij systolische HF patiënten, zowel de inspanningscapaciteit als de cardiale functie niet verbetert.

Er zijn verschillende mogelijke verklaringen voor dit resultaat. Onze bevindingen kunnen zijn beïnvloed door de selectiecriteria voor de onderzoekspopulatie. De onderzoekspopulatie bestond uit goed behandelde HF patiënten met bij aanvang relatief lage NT-proBNP spiegels. Dit kan zijn veroorzaakt doordat de patiënten geselecteerd zijn op basis van hun vermogen om inspanningstesten te ondergaan. Verder is het moeilijk om significante verbeteringen aan te tonen bij patiënten die al bij aanvang een redelijk goed inspanningsvermogen hadden. Tevens werden ernstig symptomatische patienten van hoge leeftijd met serieuze co-morbiliteiten die de oorzaak voor een beperkt inspanningsvermogen waren, niet opgenomen in deze studie. Hierdoor zijn de gevonden resultaten niet zonder meer geldig voor ernstig symptomatische HF patiënten.
Verder werden, om twee redenen, alleen patiënten met systolische HF (LVEF ≤ 0,45) in dit onderzoek geïncludeerd. De eerste reden was dat, om significante conclusies te kunnen trekken in deze betrekkelijk kleine studie, het risico om patiënten zonder HF te includeren zo klein mogelijk gehouden moest worden. Daarom zijn alleen HF patiënten met een eenduidige diagnose van HF met tekenen van systolische disfunctie toegelaten. De tweede reden is dat, zoals in de inleiding vermeld, over het algemeen de diastolische functie slechter is bij patiënten met een verminderde LVEF in vergelijking met patiënten met een behouden LVEF. Er is geen poging gedaan om patiënten met geïsoleerde diastolisch hartfalen te selecteren, waarbij immers het grootste effect van alagebrium kan worden verwacht.

Een tweede verklaring voor de resultaten van de BENEFICIAL studie kunnen de lage weefsel AGE spiegels zijn. Dit is mogelijk veroorzaakt door het lage percentage patiënten met diabetes, patiënten met redelijk goede nierfunctie en de selectie van patiënten met systolisch hartfalen. Hierdoor is het aandeel van AGE-crosslinking aan de diastolische disfunctie in deze populatie klein. Dit gegeven wordt ondersteund door de, in vergelijking met andere HF studies, lage waarden van sRAGE en het plasma AGE pentosidine.

Het ontbreken van enig effect van de AGE-breker alagebrium in de BENEFICIAL studie

wordt ook ondersteund door bevindingen beschreven in hoofdstuk 4. Bij 96 van de 102 patiënten in de BENEFICIAL studie, werden de plasma AGEs, CML en pentosidine, sRAGE en weefsel AGE spiegels bepaald. Deze spiegels werden vóór behandeling (alagebrium of placebo) en na 36 behandelweken bepaald. Alagebrium verminderde de weefsel AGEs niet. Tevens was er geen sprake van een verhoging van de plasma AGEs en de sRAGE spiegel. Deze resultaten ondersteunen daarom het onbreken van een positief effect op het inspanningsvermogen en de cardiale functie, in systolische HF patienten die behandeld zijn met alagebrium.

De voorspellende waarde van versuikerde eiwitten

Verschillende factoren, zoals LVEF, New York Heart Association (NYHA) functionele klasse, bloedarmoede en nierfunctie, zijn geïdentifieerd als onafhankelijke voorspellers van de overlevingskansen bij HF patiënten. De AGE spiegel stijgt tijdens het ouder worden, maar kan mogelijk ook een pathofysiologisch mechanisme zijn dat de verhoogde prevalentie van HF bij ouderen kan verklaren. De accumulatie van AGEs wordt, doordat het afname in elasticiteit van hart en bloedvaten veroorzaakt, gerelateerd aan het ontstaan en de progressie van HF. In slechts drie andere studies is de voorspellende waarde van plasma AGEs voor het onstaan en de progressie van HF beschreven. Onze groep toonde aan dat de plasma AGE, CML, direct gerelateerd is aan de ernst en prognose van de HF patiënt (n = 102). Deze relatie verviel echter na correctie voor de nierfuctie, maar de correlatie tussen plasma AGEs en eGFR werd aangetoond. In een ander populatie van HF patiënten (n = 141), werd een relatie aangetoond tussen serum pentosidine en de ernst van HF. Verder was serum pentosidine een onafhankelijke factor die een indicatie gaf over het risico op ongewenste klinische uitkomsten bij HF patiënten. De ongewenste uitkomsten werd gedefinieerd als een gecombineerd eindpunt van overlijden en/of ziekenhuisopname ten gevolge van HF. In de derde HF studie (n = 160), bleek pentosidine een onafhankelijke voorspeller voor ongewenste cardiale gebeurtenissen, gedefinieerd als het gecombineerde eindpunt van sterfte en/of ziekenhuisopname door HF (p = 0,012). In deze studie is CML echter niet onderzocht. In hoofdstuk 6 is aangetoond dat in een grote populatie van HF patiënten (n = 580), zowel CML als pentosidine een voorspellende factor was voor zowel ziekenhuisopname ten gevolge van HF als het gecombineerde primaire eindpunt (sterfte en/of ziekenhuisopname ten gevolge van hartfalen). Verder bleek CML significant en onafhankelijk geassocieerd met een verhoogde kans op overlijden. Deze conclusies bevestigen dat de resultaten van de drie kleinere studies van HF patiënten ook toepasbaar zijn op een grotere HF populatie. Het concept dat AGEs gerelateerd zijn aan het ontstaan en de progressie van HF wordt ondersteund door de bevindingen in dit hoofdstuk én de pathofysiologische eigenschappen.

Toekomstige vooruitzichten

Dit proefschrift beschrijft enkele nadelige klinische gevolgen van AGE-accumulatie en de effectiviteit en veiligheid van het gebruik van de AGE crosslink breker alagebrium bij chronische HF patiënten. Toekomstig onderzoek naar versuikerde eiwitten zou zich kunnen richten op drie hoofdgebieden; de schadelijke effecten van AGEs, de prognostische waarde van AGEs en de interactie tussen plasma AGEs en weefsels. In de paragrafen hieronder worden deze gebieden verder uitgediept.

Behandeling van schadelijke effecten van AGEs

De schadelijke effecten van AGE-accumulatie kunnen worden voorkomen door in te grijpen op één van de stappen van de Maillard reactie. Zoals eerder beschreven bestaat de Maillard-reactie uit zowel oxidatieve als niet-oxidatieve reactie stappen, waarbij AGEs worden gevormd door de reactie van eiwitten met suiker residuen. Om de vorming van AGEs te remmen zijn er verschillende strategieën geïndentificeerd.

De drie meest veelbelovende mogelijkheden om de de door AGEs veroorzaakte negatieve effectente reduceren, zijn hieronder weergegeven;
De eerste vermindert de AGE spiegels in het lichaam door HF patiënten te behandelen met een AGE crosslink-breker (bijv. alagebrium), met als doel de excessieve hoeveelheid door AGEs gevormde crosslinks te verminderen. Oudere HF patiënten in het algemeen, maar vooral patiënten met diabetes of nierfalen, hebben over het algemeen hoge AGE spiegels. Hierdoor zou deze patiëntengroep het meest kunnen profiteren van AGE interventie. Op dit moment wordt de AGE crosslink breker TRC 4186 onderzocht voor gebruik bij diabetische HF patiënten (eudraCT nummer van dit onderzoek is 2008-006237-27).

De tweede en derde methode probeert de reactie tussen AGE en de AGE receptor te voorkomen. De producten, die uit deze reactie ontstaan, veroorzaken cardiovasculaire disfunctie. Door deze reactie te voorkomen kan de door AGEs veroorzaakte schade worden voorkomen. Dit kan worden bereikt door sRAGE, dat AGE en de voorlopers van AGEs uit het systeem kan wegvangen, toe te voegen, of door de functionele sites van RAGE te blokkeren met antilichamen. Hoewel er voor beide methoden bemoedigende experimentele resultaten zijn gerapporteerd, zijn er nog geen klinische studies gemeld.

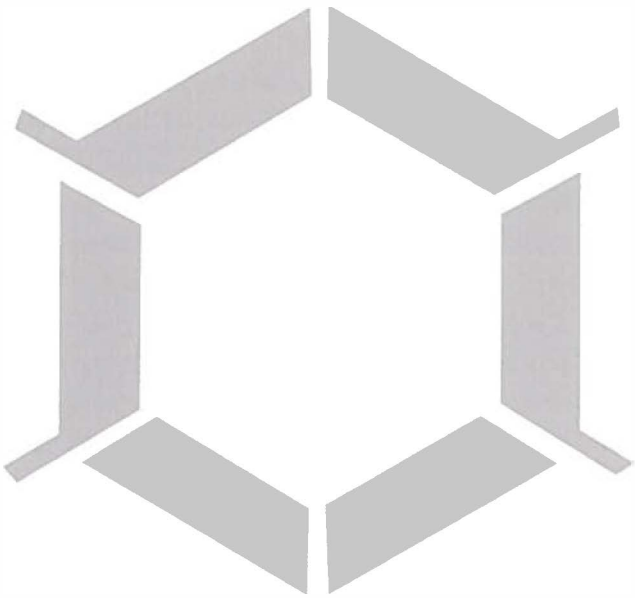
De voorspellende waarde van AGEs

AGEs kunnen, zoals eerder beschreven, ventriculaire en arteriële stijfheid veroorzaken, waardoor er een sterke relatie is met diastolische disfunctie en hartfalen. AGEs zou een pathofysiologische factor kunnen zijn voor een verhoogde prevalentie van hartfalen bij patiënten. Hoge plasma AGE spiegels (zowel CML als pentosidine) waren significant en onafhankelijk geassocieerd met een hoger sterftecijfer in patiënten opgenomen met hartfalen, vergeleken met lage plasma AGE spiegels. Er is echter geen gedetailleerde informatie beschikbaar over deze eventuele associatie voor systolisch en diastolisch HF. Echter vanuit een pathofysiologisch oogpunt is het aannemelijk dat er verschillen zijn. Systolische HF patiënten hebben excentrische hypertrofie, wat veroorzaakt kan worden door ischemie (geen AGE betrokkenheid). Diastolische HF patiënten hebben concentrische hypertrofie, wat veroorzaakt kan worden door een door AGE veroorzaakte verstijving van vertrikel en bloedvaten. Deze interactie is daarom het onderzoeken waard.

Interactie tussen weefsels en plasma AGEs

Alleen CML spiegels zijn nauwkeurig bepaald in biopten van de huid en in menselijk myocardweefsel is alleen een indicatie van de CML spiegel bepaald door middel van immunokleuringen. Bovendien is de relatie tussen huid en serum CML spiegels en die van het myocardweefsel nog niet onderzocht. Wel is er een relatie aangetoond tussen verhoogde AGE spiegels in huid en serum en een verminderde diastolische functie. Er is echt nooit vastgesteld of de verminde cardiale functie daarwerkelijk veroorzaakt wordt door de effecten van AGEs in het myocard. Mede hierom is een cross-sectionele-pilot-studie gestart naar de relatie tussen AGE spiegels van het myocard, de huid en het serum. Patiënten op de wachtlijst voor een hartoperatie zijn gevraagd mee te werken aan deze studie. Het primaire eindpunt van deze studie is de relatie tussen AGE spiegels in de huid en myocardweefsel.

Concluderend, verder onderzoek naar de effecten van AGEs is gerechtvaardigd. De volgende onderwerpen zijn interessant voor vervolgonderzoek; methoden om de negatieve effecten van AGEs te verminderen, de mogelijkheden van AGEs als voorspellers van de prognose voor HF patienten en de interactie tussen weefsel AGEs en plasma AGEs. Er blijven dus tal van mogelijkheden in een AGE-ing wereld!



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